Von Estron-Mimetika zu bicyclisch substituierten Hydroxyphenylmethanonen:

Entwicklung neuer nichtsteroidaler Hemmstoffe der 17β-Hydroxysteroid Dehydrogenase Typ 1 (17β-HSD1)

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Vorgelegt von
Alexander Oster
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Dekan:	Prof. Dr. Stefan Diebels
Berichterstatter:	Prof. Dr. Rolf W. Hartmann Jun.-Prof. Dr. Claus Jacob
Vorsitz:	Prof. Dr. Claus-Michael Lehr
Akad. Mitarbeiter:	Dr. Martin Frotscher

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Eingeflossene Publikationen

Die vorgelegte Arbeit gliedert sich in drei Publikationen, welche durch römische Zahlen gekennzeichnet sind:

I. Novel estrone mimetics with high 17β-HSD1 inhibitory activity

Alexander Oster, Tobias Klein, Ruth Werth, Patricia Kruchten, Emmanuel Bey, Matthias Negri, Sandrine Marchais-Oberwinkler, Martin Frotscher, Rolf W. Hartmann

Bioorganic & Medicinal Chemistry (**2010**) 18, 3494-3505.

II. Bicyclic substituted hydroxyphenylmethanones as novel inhibitors of 17β-hydroxysteroid dehydrogenase type 1 (17β-HSD1) for the treatment of estrogen-dependent diseases

Alexander Oster, Stefan Hinsberger, Ruth Werth, Sandrine Marchais-Oberwinkler, Martin Frotscher, Rolf W. Hartmann

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III. Bicyclic substituted hydroxyphenylmethanone type inhibitors of 17β-hydroxysteroid dehydrogenase type 1 (17β-HSD1): The role of the bicyclic moiety

Alexander Oster, Tobias Klein, Claudia Henn, Ruth Werth, Sandrine Marchais-Oberwinkler, Martin Frotscher, Rolf W. Hartmann

ChemMedChem (**2010**), Manuskript eingereicht.

Stellungnahme über die Beiträge des Autors

Der Autor möchte zu seinen Beiträgen zu den Veröffentlichungen **I–III** in der Dissertation Stellung nehmen.

I Erstellung des Hemmstoff-Designkonzeptes und Entwicklung der Hemmstoffe. Planung, Synthese und Charakterisierung aller neuen Verbindungen. Interpretation aller Ergebnisse sowie Konzipieren und Verfassen des Manuskriptes.

II Erstellung des Hemmstoff-Designkonzeptes und Entwicklung der Hemmstoffe. Planung aller neuen Verbindungen. Synthese und Charakterisierung der meisten neuen Verbindungen. Verbindungen **21**-**24** wurden von Stefan Hinsberger im Rahmen einer Diplomarbeit synthetisiert und charakterisiert. Evaluierung der Verbindungen hinsichtlich ihrer ER-Affinität. Interpretation aller Ergebnisse sowie Konzipieren und Verfassen des Manuskriptes.

III Erstellung des Hemmstoff-Designkonzeptes und Entwicklung der Hemmstoffe. Planung, Synthese und Charakterisierung aller neuen Verbindungen. Evaluierung der Verbindungen hinsichtlich ihrer ER-Affinität. Interpretation aller Ergebnisse sowie Konzipieren und Verfassen des Manuskriptes.

Weitere Publikationen des Autors, die nicht Gegenstand der Dissertation sind

- Bey, E.; Marchais-Oberwinkler, S.; Kruchten, P.; Frotscher, M.; Werth, R.; **Oster, A.**; Algul, O.; Neugebauer, A.; Hartmann, R. W., Design, synthesis and biological evaluation of bis(hydroxyphenyl) azoles as potent and selective non-steroidal inhibitors of 17β-hydroxysteroid dehydrogenase type 1 (17β-HSD1) for the treatment of estrogen-dependent diseases. *Bioorg. Med. Chem.* **2008**, 16, 6423-6435.
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- 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 non-steroidal inhibitors of 17β-hydroxysteroid dehydrogenase type 1 (17β-HSD1). *J. Med. Chem.* **2008**, 51, 6725-6739.
- Bey, E.; Marchais-Oberwinkler, S.; Negri, M.; Kruchten, P.; **Oster, 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 17β-hydroxysteroid dehydrogenase type 1 (17β-HSD1) inhibitory activity and selectivity. *J. Med. Chem.* **2009**, 52, 6724–6743.
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- Marchais-Oberwinkler, S.; Henn, C.; Möller, G.; Klein, T.; Lordon, M.; Negri, M.; **Oster, A.**; Spadaro, A.; Werth, R.; Xu, K.; Frotscher, M.; Hartmann, R. W.; Adamski, J., 17β-Hydroxysteroid dehydrogenases (17β-HSDs) as therapeutic targets: protein structures, functions, and recent progress in inhibitor development. *J. Steroid Biochem. Mol. Biol.* **2010**, in press.
- Hartmann, R. W.; **Oster, A.**; Spadaro, A.; Frotscher, M.; Marchais-Oberwinkler, S., Selective 17β-hydroxysteroid dehydrogenase type 1 inhibitors. **2010**, EP10174408.4

INHALTSVERZEICHNIS

1 EINLEITUNG

1.1 Hydroxysteroid Dehydrogenasen (HSDs)

Hydroxysteroid Dehydrogenasen (HSDs) gehören zu den Oxidoreduktasen, welche für die gegenseitige Umsetzung von Ketonen und deren korrespondierenden sekundären Alkoholen verantwortlich sind. Unter Verwendung von NADPH bzw. NAD^+ als Cofaktor katalysieren sie regio- und stereoselektiv die Oxidoreduktion in verschiedenen Positionen ihrer steroidalen Substrate (3α-, 3β-, 11β-, 17β-, 20α-, 20β-Position). Darüber hinaus sind sie in den Metabolismus verschiedener nichtsteroidaler Verbindungen involviert [Hoffmann 2007, Maser 1995, Matsunaga 2006]. HSDs, die an der Umsetzung von Steroiden beteiligt sind, spielen zentrale Rollen in der Biosynthese bzw. der Aktivierung und der Inaktivierung von Steroidhormonen.

Die klassischen Hormone werden von endokrinen Drüsen synthetisiert und ins Blut abgegeben. Nach deren Transport in das Kreislaufsystem wirken sie über einen rezeptorvermittelten Mechanismus auf eine Zielzelle. Die Lehre über diesen Ablauf bezeichnet man im Allgemeinen als Endokrinologie. 1988 wurde erstmals der Begriff der Intrakrinologie eingeführt [Labrie 1988]. Er beschreibt den Tatbestand, dass lokal produzierte Androgene und/oder Estrogene ihre Funktion in den Zellen ausüben, in denen sie synthetisiert werden, ohne dabei in den extrazellulären Raum ausgeschieden zu werden [Labrie 1991]. HSDs sind Bestandteile dieses intrakrinen Mechanismus. In den Zielzellen setzen sie inaktive Steroidhormone in die entsprechenden aktiven Formen um und umgekehrt. Somit steuern sie die intrazelluläre Besetzung der jeweiligen Steroidhormonrezeptoren [Duax 2000, Penning 1997]. Für die meisten Steroidhormone sind die dazugehörigen HSD-Enzympaare bekannt, welche die lokalen Konzentrationen der aktiven Formen und deren inaktiven Metaboliten regulieren. HSDs kann man daher als molekulare Schalter ansehen, die die Funktion der Steroidhormone modulieren können, bevor sie an den Rezeptor angreifen [Labrie 2000, Penning 2003].

Unter Berücksichtigung des intrakrinen Konzeptes wurden zahlreiche vielversprechende therapeutische Ansätze untersucht. Die Blockade von spezifischen steroidogenen Enzymen mittels potenter und selektiver Hemmstoffe trat mehr und mehr in den Fokus als Strategie zur Behandlung hormonabhängiger Erkrankungen. Dieser Ansatz wurde bereits an diversen Enzymen, die ebenfalls Steroidhormonkonzentrationen lokal beeinflussen können, erfolgreich umgesetzt. Als Beispiele können Schlüsselenzyme der Androgen- und Estrogenbiosynthese, wie

Aromatase [Gobbi 2006, Le Borgne 1997] und 5α-Reduktase [Aggarwal 2010, Baston 2002] angeführt werden.

17β-HSDs erlangten in den letzten Jahren ein Hauptinteresse als potentielle Arzneistoff-Targets für die Behandlung von sexualsteroidhormonabhängigen Krankheiten. Zwei Vorteile bietet die Gruppe der 17β-HSDs dabei: zum einen ihre gewebsspezifische Expression, zum anderen die Tatsache, dass sie oftmals die letzte Stufe der Steroidhormonbiosynthese katalysieren. Dies führt zu der Annahme, dass eine Hemmung dieser Enzyme, im Vergleich zu den vorher genannten Ansätzen (z.B. Hemmung der Aromatase), zu weniger Nebenwirkungen führen sollten, da eine geringere Beeinflussung der systemischen Steroidhormonkonzentration erwartet wird.

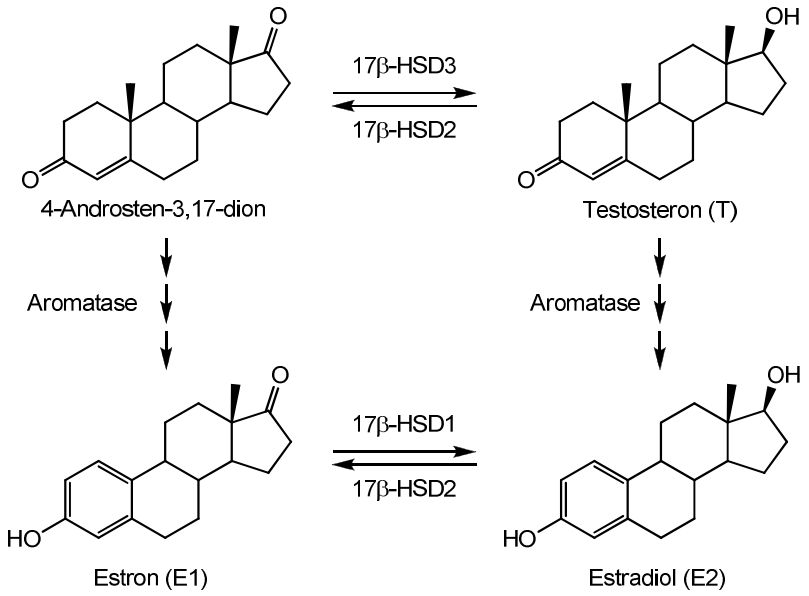
1.2 17β-HSDs: Funktionelle Aspekte

1.2.1 17β-HSD Subtypen und Multifunktionalität

Mindestens 14 unterschiedliche 17β-HSDs wurden bis heute bereits identifiziert [Luu-The 2001, Moeller 2009], von denen 12 Subtypen in menschlichem Gewebe nachgewiesen werden konnten (17β-HSD6 und 9 konnten nur bei Nagetieren nachgewiesen werden) [Moeller 2009]. Bis auf die 17β-HSD5 (zählt zu den Aldo/Ketoreduktasen AKRs) gehören alle 17β-HSDs zu der Superfamilie der SDRs (short-chain Dehydrogenasen/Reduktasen) [Lukacik 2006, Mindnich 2004]. Die verschiedenen Isoformen der 17β-HSDs besitzen eine relativ geringe Aminosäurensequenzidentität (25-30 %) [Lukacik 2006].
Sie unterscheiden sich weiterhin in ihrer Gewebeverteilung, ihrer subzellulären Lokalisation und ihrer katalytischen Präferenz (Oxidation oder Reduktion mittels Cofaktor NAD(H) oder NADP(H)).

17β-HSDs haben unterschiedliche Substratspezifitäten. *In vitro* können alle Subtypen Steroidhormone transformieren. *In vivo* hingegen sind nur 17β-HSD1, 2 und 3 in den Sexual-Steroidmetabolismus involviert und akzeptieren hier ausschließlich Steroide als Substrate [Moeller 2006, 2009].

Abbildung 1: Die Rolle von 17β-HSD1, 2 und 3 in der Steroidbiosynthese



Alle anderen Subtypen sind multifunktional und spielen eine wichtige Rolle in verschiedenen metabolischen Abläufen [Moeller 2006, 2009]. Die konkrete physiologische Rolle der jeweiligen Enzyme ist nicht restlos geklärt, wobei der momentane Wissensstand folgende Zuordnungen postuliert [Marchais-Oberwinkler 2010a]:

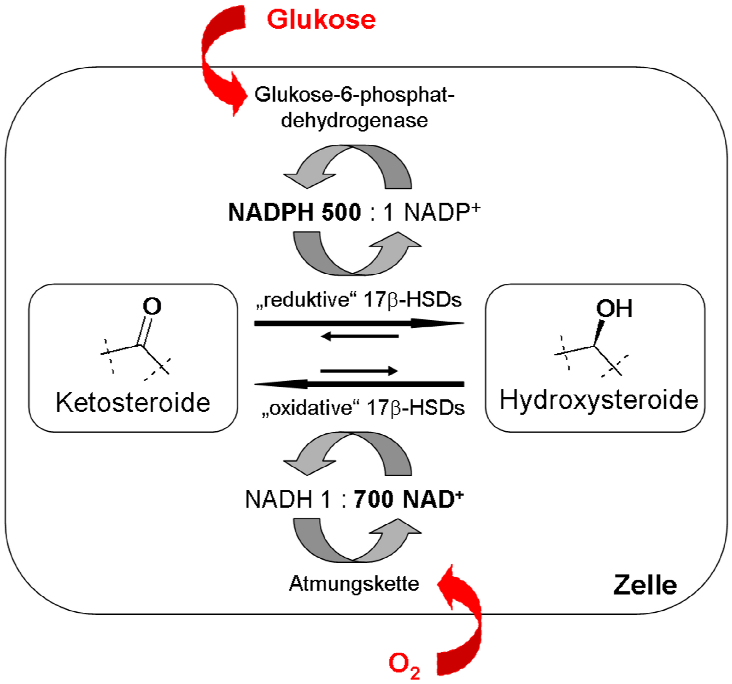
- 17β-HSD4: Fettsäureumsetzung
- 17β-HSD5: Prostaglandinsynthese
- 17β-HSD6: Retinol-Metabolismus
- 17β-HSD7: Cholesterol-Synthese
- 17β-HSD8: Fettsäureumsetzung
- 17β-HSD10: Informationsverarbeitung im Gehirn (v.a. Alzheimer-Erkrankung)
- 17β-HSD11: Lipid-Metabolismus
- 17β-HSD12: Fettsäureumsetzung
- 17β-HSD13: Retinol-Metabolismus
- 17β-HSD14: steroidogene Funktion

1.2.2 Gerichtete Katalysereaktion

Prinzipiell sind alle Vertreter der 17β-HSDs in der Lage, beide Reaktionsrichtungen (Oxidation und Reduktion) zu katalysieren. Intrazellulär zeigen sie jedoch eine klare Präferenz zu einer Katalyserichtung und werden somit in oxidative und reduktive Enzyme unterteilt [Miettinen 1996]. Verantwortlich dafür ist die Konzentration der Cofaktor-Redoxformen in der Zelle. Hier liefert NADPH die Elektronen für reduktive Reaktionen und liegt in einer millimolaren Konzentration vor, da die kontinuierliche Regeneration aus $NADP^+$ über den

Pentosephosphatweg gewährleistet ist. Von NADH und $NADP^+$ sind dagegen intrazellulär lediglich micromolare Konzentrationen vorhanden. NAD^+, das homöostatisch über die Atmungskette erneuert wird, steht als Elektronenakzeptor wiederum für oxidative Reaktionen in millimolaren Mengen zur Verfügung [Williamson 1967]. Die genauen Verhältnisse betragen etwa: NADPH zu $NADP^+$ > 500 und NAD^+ zu NADH > 700 [Agarwal 2005, Sherbet 2007].

Abbildung 2: *Determinierung der Präferenz der Katalyserichtung*



Ein weiterer Grund für die Determinierung der Katalyserichtung der 17β-HSDs ist ihre hohe Bindungsaffinität zum phosphorylierten bzw. nicht-phosphorylierten Nikotinamid-Adenin-Dinukleotid (NAD) Cofaktor [Sherbet 2007]. Durch Analysen der Röntgenkristallstrukturen sowie durch Mutagenesestudien wurde gezeigt, dass in der N-terminalen Region des Rossman folds [Buehner 1973] von 17β-HSD1 und 17β-HSD3 (reduktive 17β-HSDs) eine positiv geladene Aminosäure (17β-HSD1, Arg37 [Huang 2001]; 17β-HSD3, Arg80 [McKeever 2002]) vorhanden ist, die eine Salzbrücke mit dem 2´-Phosphatrest des NADPHs ausbilden kann. Dadurch wird die Affinität zum phosphorylierten Cosubstrat stark erhöht. Bei 17β-HSD2 und 17β-HSD4 (oxidative 17β-HSDs) ist dieses Arginin durch eine negativ geladene Aminosäure (17β-HSD2, Glu116 [Sherbet 2009]; 17β-HSD4, Asp40 [Lukacik 2010]) ersetzt. Während die Ladung des 2´-Phosphatrestes der phosphorylierten Cofaktorform abgestoßen würde, erhöht sich die Affinität zur nichtphosphorylierten Form durch die Möglichkeit mit der 2´-OH Funktion des NAD^+ Wasserstoffbrücken auszubilden.

1.3 17β-HSD1

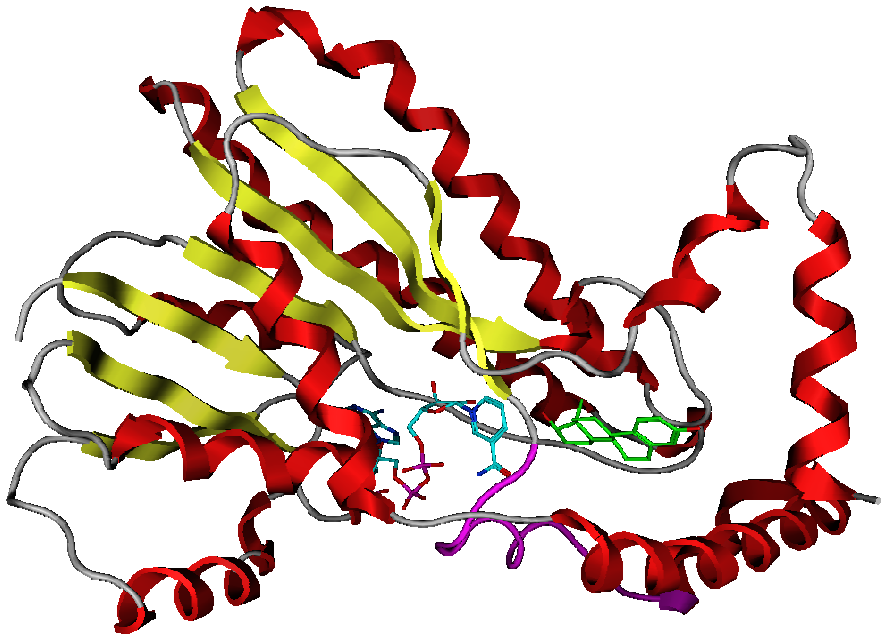
1.3.1 Struktureller Aufbau

Humane 17β-HSD1 ist als lösliches cytosolisches Homodimer aktiv [Lin 1992]. Beide Untereinheiten des Homodimers haben ein Molekulargewicht von 34,9 kDa und bestehen aus 327 Aminosäuren [Peltoketo 1988]. Zum jetzigen Zeitpunkt stehen 20 Kristallstrukturen der 17β-HSD1 in der Protein Data Bank (PDB) [Berman 2000] zur Verfügung. Diese sind dargelegt als: Apoform (1BHS), Holoform (1FDV, 1QYV), binärer Komplex mit E2, Androgenen oder Inhibitoren (1FDS, 1FDW, 1DHT, 3DHE, 1JTV, 1IOL, 3DEY, 1I5R, 3HB4, 3KLM) und ternärer Komplex mit Cofaktor und E2 oder Inhibitoren (1FDT, 1EQU, 1FDU, 1A27, 1QYW, 1QYX, 3HB5). Unter all diesen Strukturen existiert keine mit dem natürlichen Substrat E1.

Analysen der Kristallstrukturen ergaben genaue Einblicke in den strukturellen Aufbau der 17β-HSD1:

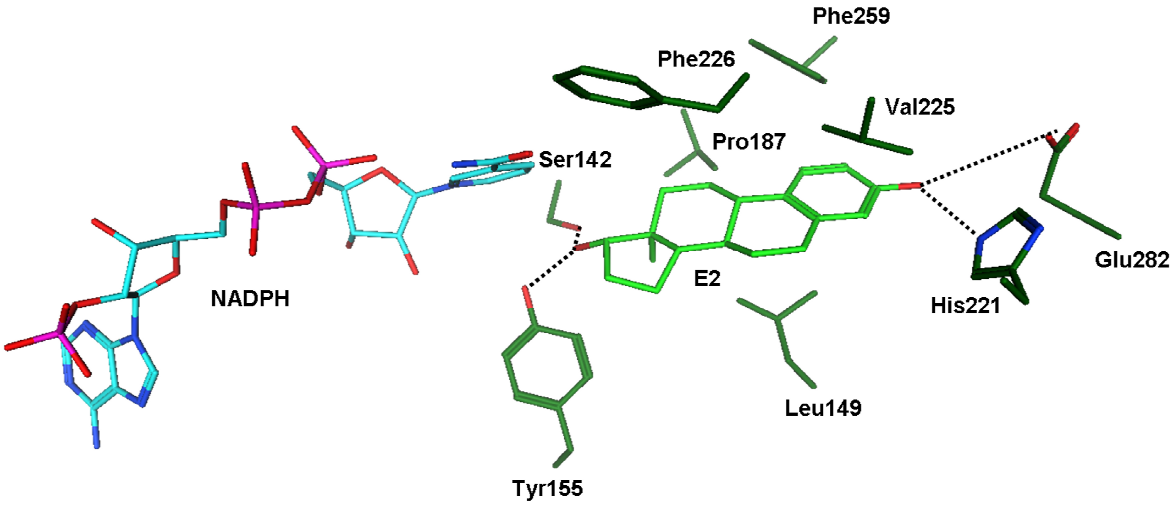
Die monomere Struktur der 17β-HSD1 besteht aus sieben parallelen β-Strängen, die zusammen ein β-Faltblatt formen, sowie aus elf α-Helices [Breton 1996, Ghosh 1995]. Das β-Faltblatt ist auf beiden Seiten von drei parallelen α-Helices umgeben. Diese Anordnung bildet den typischen „Rossman fold“, eine hochkonservierte Region, die man bei allen Mitgliedern der SDR Superfamilien findet [Jornvall 1995]. Hier findet die Bindung des Cofaktors NAD(P)H statt. Weiterhin enthält das Enzym einen hoch flexiblen βFαG´-Loop (von Pro187 bis Pro200), der, basierend auf der Präsenz von Cofaktor und Liganden, verschiedene Konformationen einnehmen kann: eine offene, eine halb-offene und eine geschlossene Konformation, die die Cofaktor- und Substratbindetasche voneinander abgrenzt [Negri 2010]. Die Rolle des Loops wird im Zusammenhang mit der $NADP^+$ Stabilisation [Mazza 1998] und dem Eintritt des Substrats ins Enzym gesehen. Stark abhängig von seiner Konformation wird dem Loop auch eine wichtige Rolle in der Bindung von Inhibitoren zugeordnet [Negri 2010].

Abbildung 3: *3D-Struktur des humanen 17β-HSD1-Monomers (PDB-ID: 1A27), cokristallisiert mit E2 (grün) und NADPH (türkis). Farbliche Markierung der Aminosäuren: gelb: β-Stränge, rot: α-Helices, magenta: flexibler Loop*



Die Ligandbindetasche ist ein enger hydrophober Tunnel, der eine hohe Komplementarität zum Substrat aufweist, sowohl was die Struktur als auch das Volumen betrifft. Als Mitglied der SDR-Familie hat die 17β-HSD1 auch eine hoch konservierte und zur Katalyse notwendige Tyr-x-x-x-Lys Sequenz und die zugehörige katalytische Tetrade (Asn114, Ser142, Tyr155, Lys159) [Filling 2002]. E2, das Produkt der katalytischen Reaktion, bindet in die „active site“ sowohl über drei bis vier Wasserstoffbrücken-Wechselwirkungen [Azzi 1996] als auch über hydrophobe Interaktionen mit den apolaren Aminosäuren der Bindetasche (Val143, Leu149, Pro187, Val225, Phe226, Phe259). Die Hydroxygruppe am C17 des Estradiols bildet Wasserstoffbrückenbindungen zu Ser142 und Tyr155 im katalytischen Zentrum. An der entgegengesetzten Seite der Ligandbindetasche interagiert His221 mit der phenolischen OH-Gruppe am C3-Atom über eine Wasserstoffbrücke. Obwohl Mutagenesestudien offenbarten, dass Glu282 keine wichtige Rolle bei der Bindung des Substrates spielt [Puranen 1997], wurden jedoch in unabhängigen Kristallstrukturen Wasserstoffbrückenbindungen zwischen dieser phenolischen Hydroxyfunktion und Glu282 identifiziert [Azzi 1996, Sawicki 1999]. Dieser Glutamatrest soll durch die Ausbildung einer Salzbrücke zum His221 auch zur Stabilität des Enzyms beitragen [Ghosh 1995].

Abbildung 4: *Bindungsmodus von E2 in der Substratbindetasche der 17β-HSD1 (PDB-ID: 1A27). Mögliche Wasserstoffbrückenbindungen sind mit schwarz gestrichelten Linien gekennzeichnet.*



1.3.2 Katalytischer Mechanismus

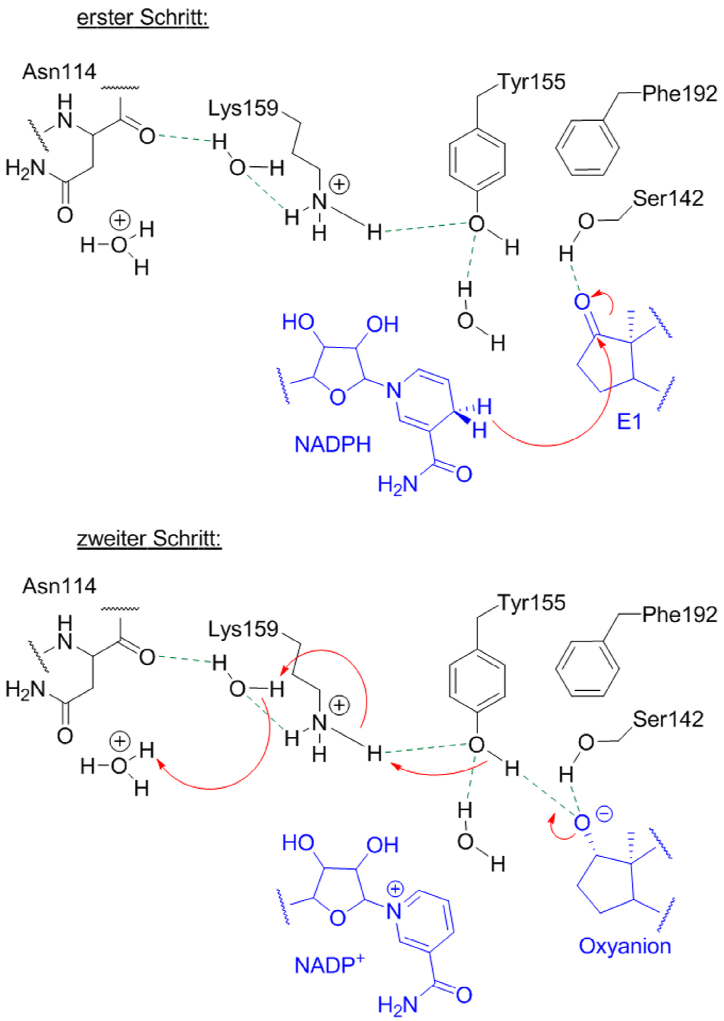
Obwohl unterschiedliche Mechanismen der Katalysefunktionen der 17β-HSDs beschrieben sind, haben sie jedoch alle folgenden chemischen Mechanismus gemeinsam: einen reversiblen Hydrid- oder Protonentransfer von NADPH oder Hydroxysteroid zum Ketosteroid bzw. zum NAD^+.

Puranen et al. berichteten erstmalig über die Beteiligung eines konservierten Wassermoleküls und der Aminosäuren Ser142, Tyr155 und Lys 159 („katalytische Triade“) an der Katalyse der 17β-HSD1 [Puranen 1994]. Weitere Forschungsergebnisse zeigten, dass ein zusätzliches hoch konserviertes Wassermolekül, welches über eine Wasserstoffbrücke mit einem Asparaginrest („katalytische Tetrade“) stabilisiert ist, eine kritische Rolle im enzymatischen Prozess des Enzyms spielt [Filling 2002]. Generell konnte aber bisher noch nicht endgültig geklärt werden, ob der von der 17β-HSD1 katalysierte enzymatische Prozess nach einem konzertierten [Ghosh 2001] oder schrittweisen [Penning 1997] Mechanismus abläuft. Wahrscheinlich ist jedoch, dass das Sauerstoffanion des tetrahedralen Kohlenstoffs am C17 in der Lage ist, besser Protonen vom Tyr155 aufzunehmen als der Sauerstoff des planaren C17-Atoms am Estron. Darum erscheint der folgende schrittweise ablaufende Mechanismus am plausibelsten:

Im ersten Schritt wird das *pro*-S Hydrid von NADPH auf das „α-face“ des planaren C17 Kohlenstoffs von E1 transferiert. Anschließend wird das daraus resultierende Sauerstoffanion von der aziden Hydroxygruppe des Tyrosins protoniert. Die Protonenübertragung wird durch verschiedene Faktoren erleichtert: ein Wasserstoffbrückennetzwerk (Lys159, zwei

Wassermoleküle und Asn114 (Carbonylsauerstoff des Rückgrats)), eine elektrostatische Wechselwirkung zwischen der protonierten Seitenkette von Lys159 und dem Phenylring von Tyr155 [Ghosh 2001] sowie durch eine T-stacking Interaktion der beiden Phenylringe von Tyr155 und Phe192 [Negri 2010]. Letzteres sollte den pK_a-Wert von Tyr155 absenken, was wiederum den zweiten Schritt beschleunigen würde.

Abbildung 5: *postulierter Katalysemechanismus. Wasserstoffbrückenbindungen sind in grün gestrichelten Linien gekenzeichnet, der Elektronentransfer in roten Pfeilen. Substrat und Cofaktor sind in blau dargestellt, Aminosäuren in schwarz.*



1.3.3 Funktion in der Biosynthese der Estrogene

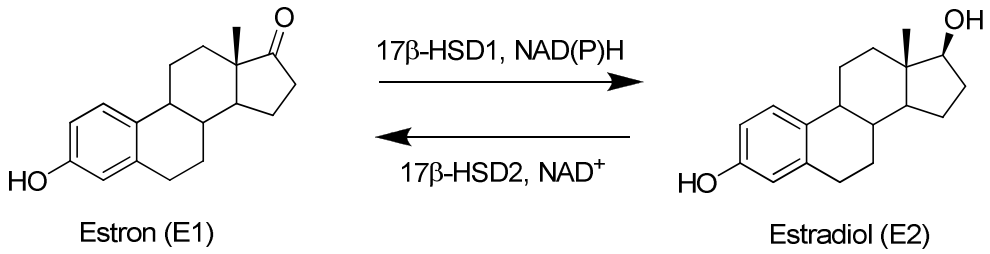
Estrogene sind weibliche Geschlechtshormone und zählen neben den Gestagenen und Androgenen zur Klasse der steroidalen Sexualhormone. Sie zeigen sowohl genitale Wirkung (z.B. Wachstumsförderung der Sexualorgane, Prägung der weiblichen Geschlechtsmerkmale, Aufbau der Uterusschleimhaut, usw.) als auch extragenitale Wirkung (z.B. Steigerung der Resorption von Kalzium sowie dessen Einlagerung in den Knochen, Vergrößerung des

subkutanen Fettdepots, usw.). Im Wesentlichen entfalten Estrogene ihre Wirkung durch Aktivierung der beiden Estrogenrezeptoren α und β (ERα, ERβ). Das aktivste Estrogen im menschlichen Organismus ist das 17β-Estradiol (E2).

Die Biosynthese der Estrogene erfolgt ausgehend vom Squalen über Cholesterol und Androgene (männliche Sexualhormone) [Ackerman 2002]. Die 17β-HSD1 nimmt in diesem biosynthetischen Ablauf eine absolute Schlüsselfunktion ein, da sie den letzten Schritt der E2-Biosynthese katalysiert: die NADPH-abhängige Reduktion der C17-Ketogruppe des schwach aktiven Estrons (E1) zur beta-ständigen Hydroxygruppe am C17 des E2 (siehe Abb. 6). Generell ist die 17β-HSD1 auch fähig, die Reduktion einiger Androgene, wie Androstendion zu Testosteron zu katalysieren. Dies geschieht jedoch nur in einem sehr geringen Ausmaß [Luu-The 1995, Poutanen 1993]. Die Gewebeverteilung im menschlichen Organismus sieht für die 17β-HSD1 eine überwiegende Expression in folgenden Geweben vor: Brust, Endometrium, Ovarien, Plazenta [Martel 1992].

Als biologischer Gegenspieler der 17β-HSD1 gilt der Subtyp 2 der 17β-HSDs [Vihko 2001]. Dieses Enzym katalysiert die Umkehrreaktion, d.h. die Oxidation und damit die Inaktivierung des hoch potenten Estradiol zum schwach aktiven Estron. Als Cofaktor dient dazu NAD^+.

Abbildung 6: gegenseitige Umwandlung von Estron (E1) und Estradiol (E2)



1.4 Estrogenabhängige Erkrankungen

1.4.1 Brustkrebs

1.4.1.1 Allgemeines

Brustkrebs ist eine der beiden häufigsten Krebsarten, die bei Frauen zum Tod führen. Es gibt verschiedene Brustkrebsarten, wobei die meisten jedoch hormonabhängiger Natur sind (z.B. Tumorzellen, die die Estrogenrezeptoren exprimieren). Es wurde bewiesen, dass Estrogene, vor allem E2, eine entscheidende Rolle bei der Entstehung und Weiterentwicklung dieser Tumore

einnehmen [Travis 2003]. Brustkrebs kann in zwei Kategorien eingeteilt werden: estrogenrezeptor-positive (ER+) und estrogenrezeptor-negative (ER-) Tumore. Etwa 50 % der Brustkrebsfälle bei prämenopausalen Frauen und 75 % bei postmenopausalen Frauen sind ER+ [Lower 1999]. Generell ist das Auftreten von Brustkrebs bei postmenopausalen Frauen häufiger, da hier die Estrogenproduktion in den Ovarien aufgehört hat, und E2 lokal in den peripheren Zielgeweben, wie beispielsweise der Brust, gebildet wird.

1.4.1.2 Therapieoptionen

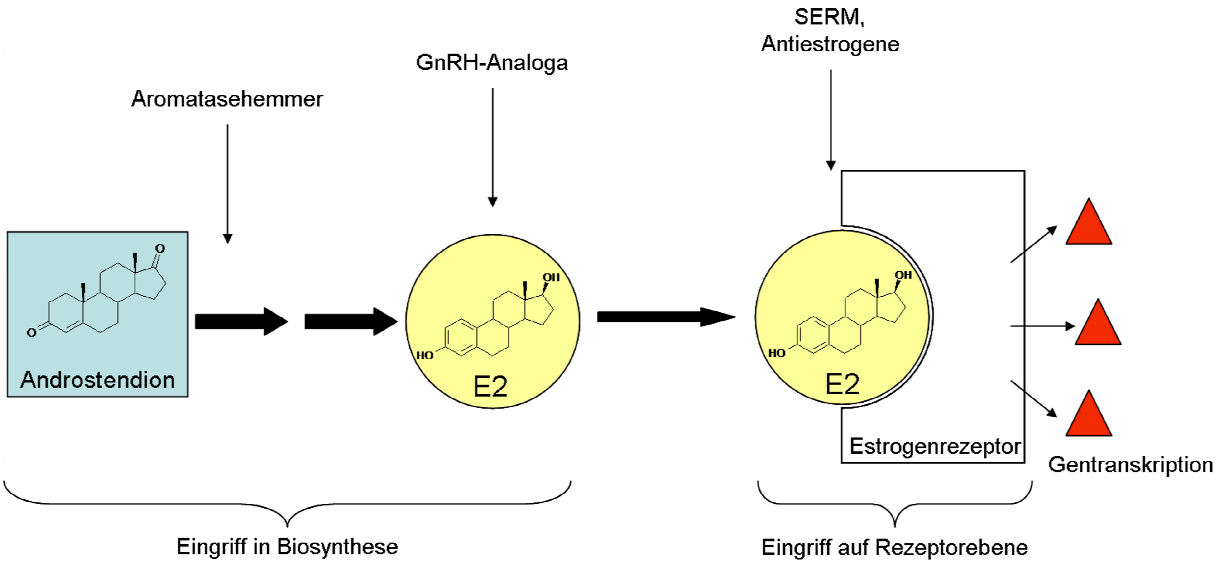
Eine oft angewendete Behandlung von Brustkrebs stellt die chirurgische Entfernung des Primärtumors dar, wobei in manchen Fällen sogar eine Mastektomie unabdingbar ist. Eine Radio- und/oder Chemotherapie kommt auch zur Behandlung in Frage, oftmals im Zusammenhang mit einer chirurgischen Entfernung des Tumors, da man den Tumor im Vorfeld auf eine operable Größe reduzieren möchte oder um nach der Operation Rezidive zu vermeiden [Fisher 2001].

Für die Behandlung estrogenabhängigen Brustkrebses stellen endokrine Therapien das Mittel der Wahl dar [Adamo 2007, Miller 2007], mit denen man versucht, die Estrogenwirkung durch ein Eingreifen in das hormonelle System zu unterdrücken [Miller 2007]. Einerseits ist dies durch die Hemmung der Estrogenbiosynthese möglich, andererseits durch die Verhinderung der Estrogenwirkung.

Folgende endokrine Therapieoptionen werden angewendet:

- Aromatasehemmer (z.B. Anastrozol):
  → Unterdrückung der Estrogenbildung durch Hemmung des letzten Schrittes der E1-Biosynthese
- GnRH-Analoga (Gonadotropin-Releasing Hormon-Analoga, z.B. Buserelin):
  → Vollständige Unterdrückung der Estrogenbildung im gesamten Organismus durch Unterbrechung eines zentralen Feedbackmechanismus [Emons 2003], der die Biosynthese steuert
- SERM (selektive Estrogenrezeptor Modulatoren, z.B. Tamoxifen):
  → Verhinderung der Estrogenwirkung am Rezeptor, wirken gewebespezifisch als Agonisten oder Antagonisten am Rezeptor
- pure Antiestrogene (z.B. Fulvestrant):
  → Verhinderung der Estrogenwirkung am Rezeptor im gesamten Organismus

Abbildung 7: Schematische Darstellung der existierenden endokrinen Therapien



1.4.2 Endometriose

1.4.2.1 Allgemeines

Endometriose ist einer der häufigsten Gründe für Unterleibsschmerzen und Unfruchtbarkeit bei Frauen. Sie ist definiert als das ektope Vorkommen von endometrialem Drüsen- und Stromagewebe. Endometrisches Gewebe wächst also dementsprechend außerhalb des Uterus, genauer gesagt vermehrt an den Ovarien, den Eileitern und in der Bauchhöhle. Es besteht jedoch keine Korrelation zwischen Ort und Größe der Läsionen und Stärke der Symptomatik. Endometriose verursacht Adhäsionen und Narben. Es kann zu starken Schmerzen, schweren Blutungen und Beschädigungen der Fortpflanzungsorgane kommen, welche letztendlich zur Unfruchtbarkeit führen können. Für die Pathogenese wurden verschiedene Theorien postuliert, jedoch konnte die Ursache bislang noch nicht abschließend herausgefunden werden. Die am weitesten verbreitete Theorie (Implantationstheorie von Sampson [Sampson 1927]) besagt, dass normale Endometriumzellen durch retrograde Menstruation in die Bauchhöhle gelangen und sich dort nach der Implantation zu Endometrioseherden weiterentwickeln. Verantwortlich für die Implantation und Proliferation des ektopen Gewebes ist das dort in einer hohen Konzentration vorliegende E2 [Bulun 2000]. In endometriotischen Läsionen ist das wachstumsfördernde E2 Bestandteil eines Teufelskreises, da es auch die Cyclooxygenase 2 (COX2) induziert [Bulun 2005], die wiederum die Produktion von Prostaglandin E2 (PGE2)

katalysiert, welches die normale Biosynthese von E2 (über die 17β-HSD1) stimuliert [Tsai 2001].

Neueste Erkenntnisse ergaben, dass die Endometrium-Hyperplasie vergleichbare Pathophysiologien aufweist [Saloniemi 2010].

1.4.2.2 Therapieoptionen

Operativ behandelt man Endometriose mittels Laparoskopie, die jedoch in der Regel lediglich für eine vorübergehende Entfernung der endometriotischen Läsionen sorgt, da die Wahrscheinlichkeit der Entstehung von Rezidiven sehr hoch ist.

Zur medikamentösen Therapie werden häufig COX-Hemmer eingesetzt, welche das entzündliche Geschehen der Endometriose verringern [Laschke 2007] und gleichzeitig für eine Schmerzlinderung sorgen.

Wie beim Brustkrebs wird auch zur Therapie von Endometriose versucht, die Estrogenbiosynthese zu unterdrücken und die E2-Produktion abzusenken. Die Applikation oraler Kontrazeptiva, Androgene und GnRH-Analoga wird dabei genutzt, um das Wachstum endometriotischer Läsionen einzudämmen, wobei auch hier Hormongleichgewichte im gesamten Organismus beeinflusst werden und es dadurch zu unerwünschten Nebenwirkungen wie z.B. Gewichtszunahme und Akne kommen kann [Berkley 2005].

1.4.3 Nachteile bestehender Therapien

Eine systemische Absenkung der Estrogenkonzentration führt zu einer Verschiebung der natürlichen Hormonbilanz im gesamten Organismus und kann daher zahlreiche bekannte, unerwünschte und zum Teil schwerwiegende Nebenwirkungen nach sich ziehen. Neben diesen Nebenwirkungen zeigen die angewendeten Therapien auch weitere Limitationen:

SERM können durch ihre gewebespezifische Funktion Krebs in anderen Geweben, wie zum Beispiel im Endometrium, in dem Tamoxifen als Estrogenrezeptor-Agonist wirkt, induzieren [DeMichele 2008, Saadat 2007]. Bei Antiestrogenen ist genau wie bei Aromataseinhibitoren eine häufige Resistenzentwicklung zu beobachten [Urruticoechea 2007]. Weiterhin können Aromatase-Hemmstoffe ausschließlich bei postmenopausalen Frauen eingesetzt werden, weil sie in prämenopausalen Frauen eine starke Stimulation der Ovarien über einen hypothalamisch/hypophysären Feedback-Mechanismus hervorrufen [Ortmann 2009].

1.5 17β-HSD1: ein neues Target zur Behandlung estrogen-abhängiger Erkrankungen?

1.5.1 Allgemeines

Estrogene spielen eine absolute Schlüsselrolle bei der Entstehung und Proliferation hormonabhängiger Erkrankungen wie Brustkrebs und Endometriose. Die 17β-HSD1 katalysiert die Umwandlung von E1 zu E2, welches das potenteste Estrogen, mit einer im Vergleich zu E1 etwa 10fach höheren Affinität zu den ERs, ist.

Gesundes Brustgewebe exprimiert sowohl die reduktive 17β-HSD1 als auch die oxidative 17β-HSD2, wobei letztere sogar etwas höher exprimiert wird [Miettinen 1999]. In Tumoren von Patienten mit ER+-Brustkrebs ist das Verhältnis von 17β-HSD1 zu 17β-HSD2 jedoch erhöht [Miyoshi 2001]. Es wurde sogar gezeigt, dass die Expression der 17β-HSD1 ein unabhängiger prognostischer Faktor für diese Erkrankung ist [Gunnarsson 2005, Oduwole 2004]. Diese gestörte Ratio zwischen den beiden 17β-HSD Subtypen findet man in ähnlichem Ausmaß bei erkranktem endometriotischem Gewebe.

Durch die erhöhte E2-Biosynthese im erkrankten Gewebe steht mehr E2 zur Verfügung, welches direkt die Proliferation stimulieren kann. Diese Überexpression des reduzierenden Enzyms spielt im Zuge des intrakrinen Regulationsprinzips eine wichtige Rolle, da eine Hemmung der 17β-HSD1 lediglich eine lokale Herabsenkung der E2-Konzentration nach sich zieht.

Weiterhin stellt die Hemmung dieses Enzyms erst ein Eingreifen in den letzten Schritt der E2-Biosynthese dar und gewährleistet somit, dass E1 im Organismus weiterhin (im Gegensatz zu anderen endokrinen Therapien) seine schwache estrogene Wirkung entfalten kann. Damit wäre dem radikalen Estrogenentzug vorgebeugt, welcher die typischen Nebenwirkungen wie Stimmungsschwankungen bis hin zur Depression, Osteoporose oder Hitzewallungen hervorruft.

Die dargestellten Gründe und die Option, dass Hemmstoffe der 17β-HSD1 auch als alternative Therapie bei z.B. einer Resistenzentwicklung gegenüber Tamoxifen eigesetzt werden könnten, rückt den Fokus auf die 17β-HSD1 als vielversprechendes Target zur Behandlung estrogenabhängiger Erkrankungen mit weniger Nebenwirkungen.

1.5.2 Tiermodelle

In verschiedenen Tiermodellen wurde bereits die physiologische Rolle der 17β-HSD1 bzw. deren Hemmung als Therapieansatz sowohl bei Brustkrebs als auch bei Endometriose untersucht und bestätigt.

Zwei Xenograft-Modelle mit Nacktmäusen wurden zur Evaluation von Hemmstoffen der 17β-HSD1 entwickelt. Die Mäuse wurden im ersten Modell [Husen 2006a, 2006b] mit MCF-7-Zellen, die rekombinante humane 17β-HSD1 exprimieren, im zweiten Modell [Day 2008a] mit der Brustkrebszelllinie T47D (exprimiert 17β-HSD1 und 2 und spiegelt somit das Gleichgewicht eines erkrankten Gewebes wider), angeimpft. In beiden Modellen konnten Hemmstoffe der 17β-HSD1 eine Hemmung des Tumorwachstums erzielen.
Ein transgenes Maus-Modell, bei dem humane 17β-HSD1 ubiquitär exprimiert wird [Lamminen 2009], wurde von Lamminen *et al.* entwickelt. Neben der Hemmung der Estronumsetzung konnte man mit diesem Modell durch die Applikation eines 17β-HSD1 Hemmstoffes auch Endometrium-Hyperplasie der transgenen Mäusen umkehren [Saloniemi 2010].
Grümmer et al. etablierten ein Endometriose-Modell [Grümmer 2001], bei dem man Nacktmäusen endometriotisches Gewebe menschlicher Spenderinnen implantierte. Hier wurden Expressionsmuster steroidumsetzender Enzyme detektiert, die von Hemmstoffen der 17β-HSD1 verändert wurden [Firnhaber 2006].
Das interessanteste Endometriose-Modell wurde mit Marmosetaffen (Callithrix Jacchus) erstellt [Einspanier 2006], deren 17β-HSD1 eine hohe Homologie zum menschlichen Enzym aufweist. Hier wurden Endometriumzellen mit Pufferlösung durch den Uterus in den Bauchraum gespült, wo sie sich zu Endometrioseherden entwickeln. Mittels 17β-HSD1-Inhibitoren sollte man die Größe der endometrischen Läsionen verringern können. Darüber hinaus kann detektiert werden, ob auch der Blutfluß innerhalb dieser Läsionen beeinflusst wird.

Zusammenfassend unterstreichen die aufgeführten Befunde die Bedeutung der 17β-HSD1 für estrogenabhängige Erkrankungen und stellen heraus, dass die Entwicklung von Hemmstoffen dieses Enzyms ein vielversprechender Ansatz zu deren Therapie ist.

1.6 Literaturbekannte Inhibitoren der 17β-HSD1

1.6.1 Allgemeines

Während der letzten Jahre ist die Anzahl an wissenschaftlichen Publikationen, die Hemmstoffe der 17β-HSD1 zum Thema haben, kontinuierlich angestiegen. Mehrfach wurden bereits auch Übersichtsartikel über 17β-HSD1 Inhibitoren und deren Fortschritte veröffentlicht [Brožic 2008, Day 2008b, 2010, Marchais-Oberwinkler 2010a, Poirier 2003, 2009]. Diese Tatsache unterstreicht das große Potential, das die pharmazeutische Industrie bzw. die Wirkstoffforschung im Allgemeinen in diesem Target sieht. Im Folgenden sind verschiedene Hemmstoffklassen kurz beschrieben, von denen jeweils ausgesuchte Vertreter und zugehörige

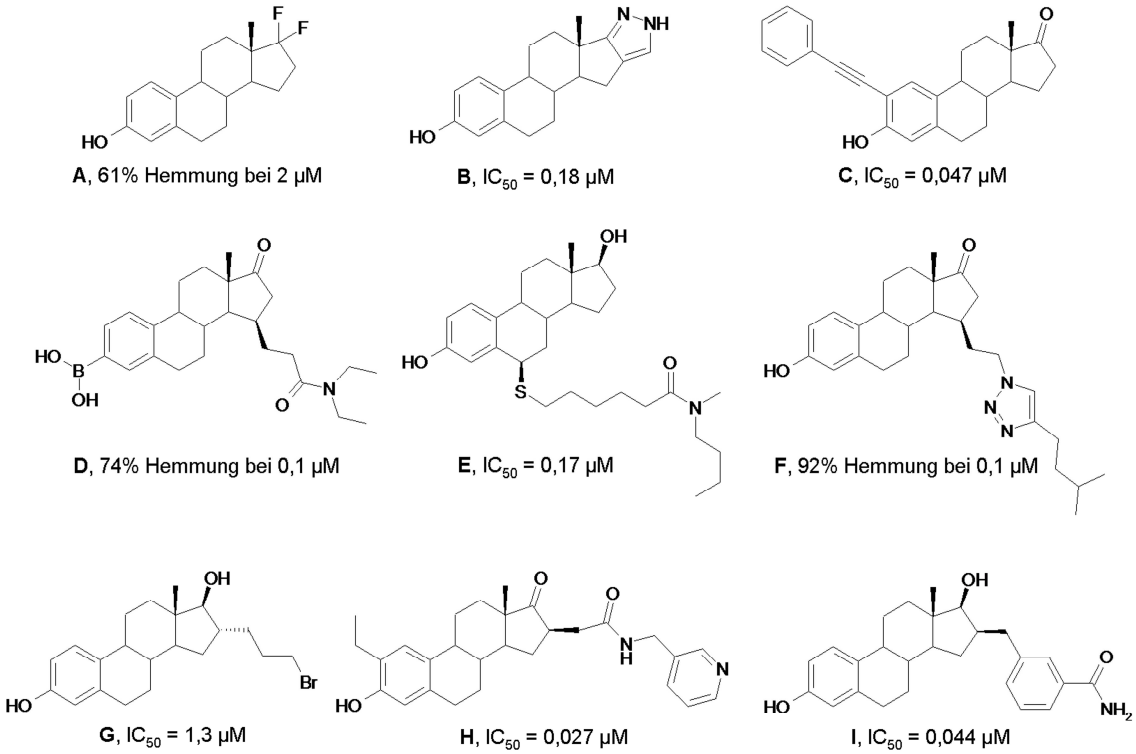
17β-HSD1 Hemmwerte als Beispiele angegeben sind. Da die meisten Hemmstoffe von verschiedenen Forschern entwickelt und biologisch evaluiert wurden, kann man deren Hemmwerte zwar bewerten, jedoch aufgrund der Verwendung unterschiedlicher Assays nicht direkt miteinander vergleichen.

1.6.2 Steroidale Inhibitoren

Die Mehrheit der literaturbekannten Hemmstoffe der 17β-HSD1 basieren auf Modifikationen eines steroidalen Grundgerüsts. Große Bibliotheken an steroidalen Hemmstoffen wurden synthetisiert, bei denen jeweils verschiedene Positionen in den Fokus gerückt wurden. Neben Variationen an Position 17, wobei man den Sauerstoff von E1 bzw. E2 durch z.B. Fluor (Verbindung **A** [Deluca 2006]) ersetzte oder das Steran-Gerüst um einen zusätzlichen E-Ring (z.B. Pyrazol, **B** [Fischer 2005]) erweiterte, wurden hauptsächlich, zum Teil auch sehr große, Seitenketten an den Positionen 2, 6, 15 und 16 eingeführt. Die *Schering AG* untersuchte intensiv den Einfluss von C2-Substituenten (z.B. längerer substituierter Alkylketten) am E1-Grundgerüst auf die Hemmung der 17β-HSD1 und erzielte sehr potente Inhibitoren mit IC_{50}-Werten im unteren nanomolaren Bereich (z.B. Verbindung **C** [Schering 2006a]). Unter Voraussetzung des steroidalen Bindungsmodus wird der Bereich nahe der C-terminalen Region des Proteins als Bindungsort für diese Substituenten vermutet. Weiterhin wurde bereits bewiesen, dass das Einführen kleiner Substituenten (z.B. Ethyl, Methoxy) in Position 2 eine verringerte Affinität der Verbindung zu den Estrogenrezeptoren nach sich zieht [Cushman 1995, Leese 2005].
Der Austausch der phenolischen OH-Gruppe am steroidalen A-Ring führte ebenfalls zu aktiven Hemmstoffen. Derivate mit einer Boronsäure-Funktion in 3-Position zeigten Hemmwerte von bis zu 74 % bei einer Inhibitorkonzentration 0,1 μM (Verbindung **D** [Solvay 2008]), wobei diese Verbindungen von ihrem Aufbau her eher Synthesezwischenstufen darstellen als ernstzunehmende Wirkstoffkandidaten.
Das Einfügen großer flexibler Seitenketten (vor allem in β-Konformation) wirkte sich in den Positionen 5, 15 und 16 am positivsten auf die Aktivität der Inhibitoren aus. Modeling Studien ergaben, dass alle flexiblen Seitenketten an diesen drei Positionen in die Cofaktorbindetasche hineinragen sollen. Während Substituenten an C5 (meist Alkylthioether, wie **E** [Poirier 1998]) nur wenig erforscht wurden, gibt es mittlerweile eine große Vielfalt an hochaktiven Derivaten mit Variationen an C15 (z.B. **F** [Solvay 2008]) und C16 (z.B. **G** [Rouillard 2008, Sam 1998]). Aus den strukturell verschiedensten, jedoch alle mit flexiblen Seitenketten substituierten, Hemmstoffen der 17β-HSD1 sind besonders zwei hervorzuheben. Zum einen wurde mit Verbindung **H** (STX1040) [Lawrence 2005] das erste „proof of concept“ durchgeführt [Day 2008a], zum anderen wurde Verbindung **I** (E2B) [Laplante 2008] kürzlich in 17β-HSD1 cokristallisiert und offenbarte dadurch, dass sich der Amidobenzylrest in eine kleine,

überwiegend lipophile, zusätzliche Bindetasche unterhalb des katalytischen Zentrums einlagert [Mazumdar 2009].

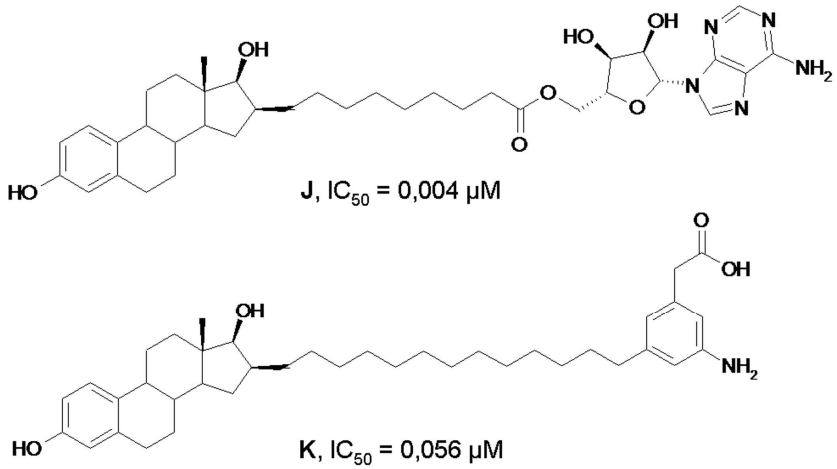
Abbildung 8: *Beispiele für steroidale Inhibitoren verschiedener Substitutionsmuster und deren biologische Daten für Hemmung der 17β-HSD1*



1.6.3 Hybridinhibitoren

Im Jahre 2002 wurden erstmalig Hybridinhibitoren beschrieben, die als sogenannte „Dual-site"-Hemmstoffe entwickelt wurden [Qiu 2002]. Diese Verbindungen sind aufgrund ihres strukturellen Aufbaus eher als wissenschaftliche Werkzeuge zu sehen denn als potentielle Wirkstoffkandidaten. Der erste hochaktive Hemmstoff **J** (EM-1745) [Qiu 2002] bestand aus einem Estradiol-Grundgerüst, welches an Position 16 über eine lange Alkylkette mit einem Adenosinrest verbunden ist, mit dem Ziel, sowohl die Steroid- als auch die Cofaktorbindetasche zu besetzen. Das Cokristallisat (PDB-ID: 1I5R) [Qiu 2002] des Hemmstoffs in der 17β-HSD1 bestätigte diesen Bindungsmodus. Kürzlich wurde auch das entsprechende äquipotente Estron-Derivat publiziert [Berube 2009b]. Weitere Nachforschungen zur Vereinfachung der Adenosin-Struktur führten zu ähnlichen, jedoch schwächer aktiven, Substanzen, bei denen der Adenosin-Rest durch z.B. carboxy- und aminosubstituierte Phenylringe (Verbindung **K** [Berube 2009a]) ersetzt wurde.

Abbildung 9: *EM-1745 und Beispiel für ein vereinfachtes Hybridinhibitor-Derivat und deren biologische Daten für Hemmung der 17β-HSD1*



1.6.4 Nichtsteroidale Inhibitoren

Auf dem Gebiet der nichtsteroidalen Inhibitoren wurden vergleichsweise weniger Hemmstoffe der 17β-HSD1 veröffentlicht. Im Gegensatz zu den steroidalen Strukturen könnten nichtsteroidale Verbindungen diverse Vorteile, wie z.B. Selektivität gegenüber steroidumsetzenden Enzymen aufweisen.

Als erste 17β-HSD1 Hemmstoffe wurden Phytoestrogene wie z.B. Coumestrol **L** [Makela 1995] und Gossypol-Derivate, welche in die Cofaktorbindetasche binden (z.B. Verbindung **M** [Brown 2003]), beschrieben. Der Nachteil beider Verbindungsklassen ist ihre bekannte, sehr geringe Selektivität, da sie auch zahlreiche andere Enzyme hemmen.

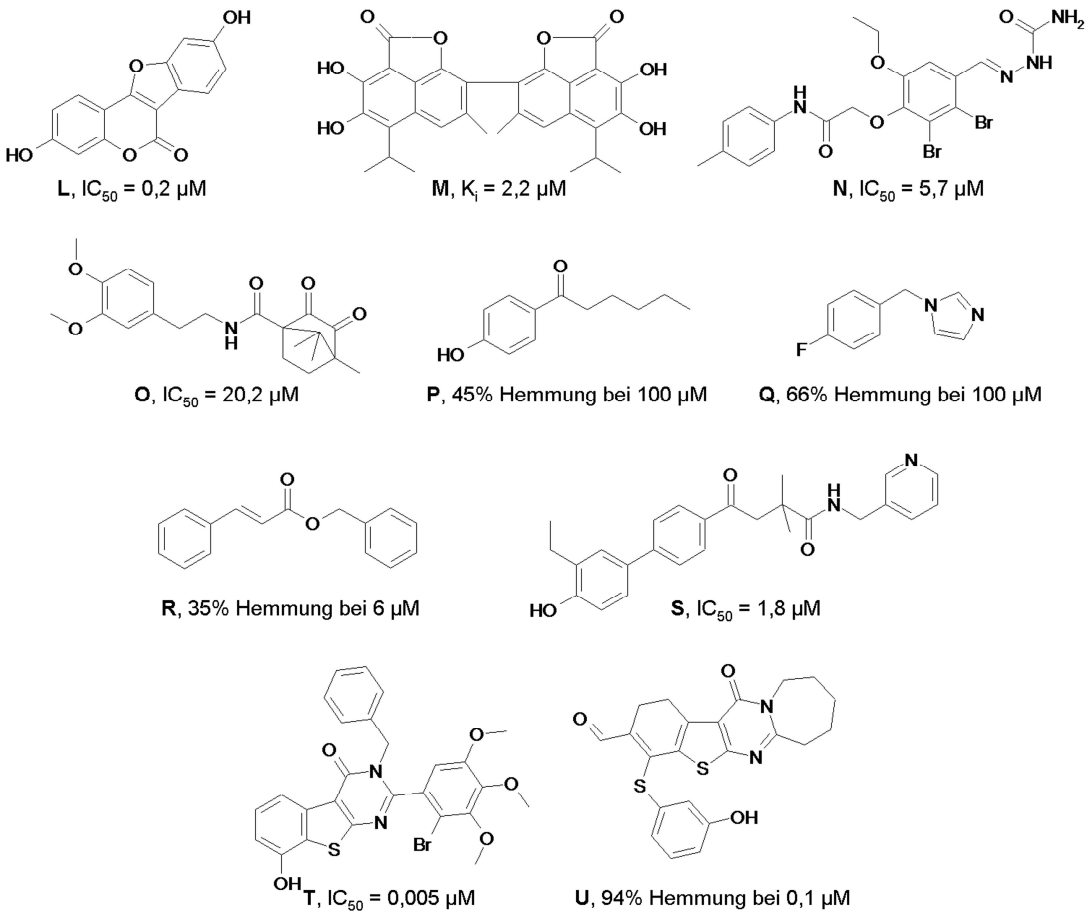
Lediglich geringe Hemmaktivität zeigten 17β-HSD1 Inhibitoren (**N** [Schuster 2008] und **O** [Schuster 2006]), die aus vorhandenen Substanzbibliotheken durch computergestützte Verfahren mittels unterschiedlicher Pharmakophor-Modelle von Schuster et al. entdeckt wurden. Ähnlich schwach aktive Substanzen fand man sowohl in Phenylketonen (z.B. **P** [Lota 2007]) und Phenylalkylimidazolen (z.B. **Q** [Olusanjo 2008]) als auch in Zimtsäureester-Derivaten (z.B. **R** [Kocbek 2010]), die ursprünglich als Hemmstoffe der 17β-HSD1cl (aus Pilz *Cochliobolus lunatus*) entwickelt wurden.

Mit dem Ziel, den natürlichen Liganden Estron zu imitieren, wurden Biphenylethanone und Indanon/Tetralon-Derivate, zum Teil mit literaturbekannten Seitenketten (z.B. **S** [Allan 2008]), erforscht, welche eine mittlere Aktivität aufzeigten.

Eine sehr große Hemmstoffklasse der 17β-HSD1 stellen die Thiophenpyrimidinone dar. Generell kann man sie in zwei Generationen einteilen. Die ersten potenten Vertreter wurden von Messinger et al. beschrieben und enthielten im Gegensatz zu der zweiten Generation drei

statt vier ankondensierte Ringe als Grundgerüst. Als aktivste Vertreter gingen Verbindung **T** [Messinger 2006] für die alte Generation und das 3-Hydroxyphenyl-Derivat **U** [Karkola 2008] hervor, welche bis heute zwei der aktivsten 17β-HSD1 Hemmstoffe sind.

Abbildung 10: Beispiele für nichtsteroidale Inhibitoren verschiedener Substanzklassen und deren biologische Daten für Hemmung der 17β-HSD1



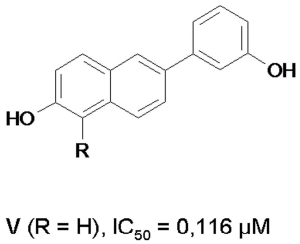
1.6.5 Nichtsteroidale Inhibitoren des Arbeitskreises Hartmann

Alle Verbindungen, die im Arbeitskreis von Prof. Hartmann entwickelt wurden, werden in einem biologischen Screeningsystem evaluiert, das aus 10 unterschiedlichen Assays für Aktivität (zellular, zellfrei), Selektivität (17β-HSD2, ERα, ERβ, Proliferationsassay, CYP-Enzyme) und Bestimmung des *in vivo*-Profils der Verbindungen besteht [Kruchten 2009c]. Neben der Evaluierung der Verbindungen hinsichtlich Aktivität an anderen Spezies [Kruchten 2009b] wurde in diesem Arbeitskreis auch ein *in vitro* proof of concept mit ausgesuchten Verbindungen unten genannten Klassen erbracht, die eine E1-induzierte T47D-Zellproliferation hemmten [Kruchten 2009a].

Unser Arbeitskreis publizierte (Hydroxyphenyl)naphthalene und –chinoline, unter denen das 6-(3-Hydroxyphenyl)-2-naphthol **V** [Frotscher 2008] die beste inhibitorische Wirkung zeigte. Zum Einfügen passender Substituenten wurden nahezu alle Positionen der Verbindung **V** untersucht, wobei sich die Position 1 als ideal herausstellte. Durch das Einfügen eines Phenylringes **W** [Marchais-Oberwinkler 2008, 2009] konnte nicht nur das inhibitorische Profil verbessert werden. Es wurde auch eine deutliche Steigerung der Selektivität gegenüber den Estrogenrezeptoren α und β erzielt. Molekulare Docking Studien ergaben einen E2-imitierenden Bindungsmodus, bei dem der Substituent in Position 1 in Richtung des Cofaktors NADPH zeigt. Durch weitere Strukturvariationen an dieser Position wurden weitere 17β-HSD1 Hemmstoffe entwickelt, die neben einem geeigneten inhibitorischen auch ein gutes pharmakokinetisches und toxikologisches Profil besitzen. Der beste Vertreter dieser Studie ist Verbindung **X** [Marchais-Oberwinkler 2010b], welche eine gute Pharmakokinetik nach peroraler Applikation und eine hohe metabolische Stabilität aufweist.

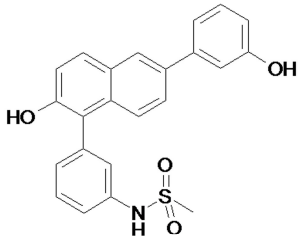
Eine zweite Hemmstoffklasse beinhaltet die Bis(hydroxyphenyl)arene [Al-Soud 2009, Bey 2008a, 2008b, 2009,]. In einer ausführlichen SAR (Struktur-Wirkungsbeziehung)-Studie wurde unter anderem herausgefunden, dass in dieser Klasse der Austausch des mittleren Ringes eine völlig inaktive Verbindung in einen hochaktiven und selektiven Hemmstoff umwandeln kann [Bey 2008b]. Einen möglichen Grund für die beobachteten scharfen Struktur-Wirkungsbeziehungen fand man in den unterschiedlichen MEP (Molekular-Elektrostatisches Potential)–Verteilungen dieser Verbindungen. Die Tatsache, dass eher kleine Substituenten (vor allem Fluor) und deren Positionen im Molekül einen großen Einfluss auf die Hemmaktivität hatten, unterstützte diese Theorie. Das Einfügen eines Fluorsubstituenten (Verbindung **Y** [Bey 2009]) führte im Vergleich zu dessen unsubstituierten Analogon **Z** [Bey 2008b] zu einer Steigerung der Aktivität und Selektivität. Zwei plausible Bindungsmodi wurden durch Docking Studien entdeckt: ein steroidaler Bindungsmodus und ein alternativer, bei dem der Hemmstoff zum Cofaktor hin verschoben ist [Bey 2009].

Abbildung 11: Beispiele für nichtsteroidale Inhibitoren des Arbeitskreises Hartmann und deren biologische Daten für Hemmung der 17β-HSD1

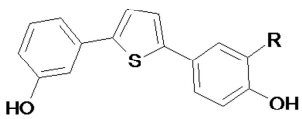


V (R = H), IC_{50} = 0,116 µM

W (R = Phenyl), IC_{50} = 0,020 µM



X, IC_{50} = 0,015 µM



Y (R = F), IC_{50} = 0,008 µM

Z (R = H), IC_{50} = 0,069 µM

2 Ziel der Arbeit

2.1 Wissenschaftliches Ziel

Estrogene sind ein wichtiger Faktor bei der Entstehung und Progression estrogenabhängiger Erkrankungen. Eine Verminderung der Estrogenkonzentration bzw. eine Verringerung der estrogenen Wirkung am Rezeptor stellt eine oft angewandte Therapieoption solcher Erkrankungen dar. Dies ist allerdings mit zahlreichen Nebenwirkungen verbunden, welche auf der Tatsache basieren, dass die estrogene Aktivität im gesamten Organismus radikal abgesenkt wird.

Ein neuer Ansatz zur Therapie solcher hormonabhängiger Erkrankungen ist die Hemmung der 17β-HSD1, welche die Umsetzung des schwach aktiven E1 in das potenteste Estrogen E2 katalysiert und somit für die Umsetzung des letzten Schrittes der E2-Biosynthese verantwortlich ist. Bei dieser Therapie bleibt im gesamten Organismus zumindest das schwach aktive E1 erhalten. Dadurch wird ein vollständiger Estrogenentzug und damit die Ursache der Nebenwirkungen der etablierten Therapien vermieden.

Ein weiterer Vorteil dieses Ansatzes beruht auf der selektiven Exprimierung der 17β-HSD1 in den erkrankten Geweben. Im Zuge einer intrakrinen Therapie sollte man durch die Hemmung dieses Enzyms die E2-Konzentration in den betroffenen Zellen lokal absenken und damit die Proliferation der Krankheit therapieren können.

Die Effektivität von Hemmstoffen der 17β-HSD1 wurde bereits in unterschiedlichen *in vivo*-Assays bewiesen [Husen 2006a, 2006b, Saloniemi 2010] und unterstreicht damit die Relevanz dieser neuartigen, vielversprechenden Therapieform. Trotz dieser Erkenntnisse und der relativ hohen Anzahl an Inhibitoren, die in den letzten Jahren publiziert wurden, gelang es bisher mit keiner Substanz, in die klinische Entwicklung zu gelangen.

Der Anspruch dieser Arbeit ist es, neue Hemmstoffe der 17β-HSD1 zu entwickeln, die alle Voraussetzungen erfüllen, die das therapeutische Konzept voraussetzt. Folgende Anforderungen werden an gute 17β-HSD1 Inhibitoren gestellt:

- hohe Aktivität gegenüber dem eigentlichen Enzym
- hohe Selektivität gegenüber dem biologischen Gegenspieler 17β-HSD2, welcher die Umkehrreaktion (Inaktivierung des E2 zu E1) katalysiert
- möglichst geringe Affinität zu den Estrogenrezeptoren α und β (ERα und ERβ), um intrinsische estrogene Effekte zu vermeiden

- hohe intrazelluläre Aktivität in T47D Zellen (Krebszelllinie)

Weiterhin sollte die metabolische Stabilität der potentiellen Wirkstoffkandidaten an humanen Lebermikrosomen bestimmt werden, um eine schnelle Phase 1-Metabolisierung auszuschließen. Alle Hemmstoffe sollen mit dem Fernziel entwickelt werden, in *in vivo*-Krankheitsmodellen getestet zu werden und Kandidaten für den Eintritt in die klinische Entwicklung darzustellen.

2.2 Strategie

In der angewendeten Design-Strategie werden steroidale Strukturen vermieden, um die Gefahr von unerwünschten Nebenwirkungen, verursacht durch Interaktionen mit Steroidhormonrezeptoren, zu verringern.
In unserem Arbeitskreis wurden bereits zwei unabhängige Substanzklassen von nichtsteroidalen Inhibitoren der 17β-HSD1 entwickelt (siehe Kapitel 1.6.5), welche beide zwei Hydroxygruppen tragen, die die beiden hydrophilen Gruppen des natürlichen Substrats E1 imitieren. Eine *meta*-Hydroxyphenyl-Teilstruktur stellte sich in beiden Klassen als absolute Voraussetzung für hohe inhibitorische Aktivität heraus und wird auch in weiteren Designstrategien eine wichtige Rolle spielen. Unser ligandbasierter Ansatz orientiert sich ebenfalls an E1, welches in dieser Arbeit als Templat benutzt wird. Der steroidale A-Ring soll durch eine *meta*-OH-Phenylstruktur ersetzt werden. Unterschiedliche Heterocyclen oder funktionelle Gruppen mit purer Wasserstoffbrückenakzeptorqualität (im Gegensatz zur OH-Gruppe der in Kapitel 1.6.5 beschriebenen Substanzklassen) sollen die Ketogruppe des steroidalen D-Ringes imitieren. Um den lipophilen Charakter der steroidalen Grundstruktur nachzuahmen, werden die beiden genannten hydrophilen Elemente des Inhibitors durch einen hydrophoben Kern verbunden.
Durch Mutagenesestudien und vor allem durch das Untersuchen der zahlreichen verfügbaren Kristallstrukturen der 17β-HSD1 haben wir bereits sehr detaillierte Einblicke in den strukturellen Aufbau und die molekularen Zusammenhänge innerhalb des Enzyms gewonnen. Diese Erkenntnisse sollen in das Design neuer Inhibitoren mit einbezogen werden. Durch den ligandbasierten Ansatz werden daher Wasserstoffbrückenbindungen der *meta*-OH-Phenyl-Teilstruktur mit den Aminosäuren His221 und Glu282 nahe der C-terminalen Region erwartet. Der D-Ring imitierende Part ragt zur katalytischen Tetrade und bildet dort Wasserstoffbrücken mit Ser142 und Tyr155 aus.
Durch Molecular Modelling Studien sowie durch genaue Struktur-Wirkungsbeziehungen sollen die designten Inhibitoren weiterentwickelt werden, vor allem hinsichtlich einer Optimierung der vermuteten hydrophilen Wechselwirkungen und einer Erschließung weiterer bislang unbeachteter bzw. ungenutzter Regionen des Enzyms oder speziell der Bindetasche.

Die vorliegende Arbeit soll zunächst den Fokus auf das Design und die Entwicklung einer neuen Substanzklasse von heterocyclisch substituierten Biphenylolen richten, deren verschiedene Heterocyclen im katalytischen Zentrum wechselwirken und sowohl dort als auch in der C-terminalen Region die Interaktionen des natürlichen Substrates E1 imitieren sollen. Als verbindende Einheit wird ein Benzolkern verwendet, um die Wechselwirkungen des Steroids mit dem hydrophoben Tunnel einzugehen. Weiterhin soll dieser Kern durch Pyridin ausgetauscht werden, um zusätzliche Interaktionen mit hydrophilen Aminosäuren (z.B. Asn152, Tyr218 oder Ser222) in der überwiegend hydrophoben Bindetasche zu ermöglichen. Molecular Modelling Studien sollen Einblick über die Bindungsmodi der einzelnen Inhibitoren geben und in weitere Designstrategien mit einbezogen werden.

3 Ergebnisse

3.1 Novel Estrone Mimetics with high 17β-HSD1 Inhibitory Activity

Alexander Oster, Tobias Klein, Ruth Werth, Patricia Kruchten, Emmanuel Bey, Matthias Negri, Sandrine Marchais-Oberwinkler, Martin Frotscher, Rolf W. Hartmann

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Bioorganic & Medicinal Chemistry, 18 (**2010**) 3494-3505

Publikation I

Abstract

17β-hydroxysteroid dehydrogenase type 1 (17β-HSD1) catalyzes the reduction of estrone into estradiol, which is the most potent estrogen in humans. Lowering intracellular estradiol concentration by inhibition of this enzyme is a promising new option for the treatment of estrogen dependent diseases like breast cancer and endometriosis. Combination of ligand- and structure-based design resulted in heterocyclic substituted biphenylols and their aza-analogs as new 17β-HSD1 inhibitors. The design was based on mimicking estrone, especially focusing on the imitation of the D-ring keto group with (substituted) heterocycles. Molecular docking provided insights into plausible protein-ligand interactions for this class of compounds. The most promising compound **12** showed an inhibitory activity in the high nanomolar range and very low affinity for the estrogen receptors α and β. Thus, compound **12** is a novel tool for the elucidation of the pharmacological relevance of 17β-HSD1 and might be a lead for the treatment of estrogen dependent diseases.

Introduction

Estrogens, especially the most active one estradiol (E2), are well known to be responsible for the development of estrogen dependent diseases like breast cancer[1] and endometriosis.[2] Current endocrine therapies for breast cancer are either focused on blocking the estrogen action at the

receptor level by selective estrogen receptor modulators (SERMs) and pure antiestrogens[3] or on decreasing the formation of estrogens by application of GnRH analogs and aromatase inhibitors.[4, 5] In case of the latter compounds intensive efforts in the last two decades[6, 7] resulted in therapeutics which – according to the FDA guidelines – are first-line therapeutics for the treatment of breast cancer.[7] However, all of these therapeutic approaches show disadvantages by causing side effects due to their rather radical reduction of systemic estrogen concentration.

A more sophisticated approach is focused on the hormone concentration in the target cell (intracrine approach). This strategy has been pursued for androgen dependent diseases for quite some time. Steroidal[8] and later non-steroidal[8-12] 5α-reductase inhibitors have been developed and two of the former are used clinically today.[8] In case of estrogen-dependent diseases a promising target came up, 17β-hydroxysteroid dehydrogenase type 1 (17β-HSD1). This enzyme catalyzes the reduction of the weakly active estrone (E1) to yield the highly active E2 using NAD(P)H as cofactor, a reaction which represents the last step in E2 biosynthesis (Chart 1). Although the subtypes 17β-HSD7 and 12 are also able to catalyze this conversion, their main physiological roles are supposed to be restricted to cholesterol biosynthesis[13] and regulation of lipid biosynthesis[14] respectively.

17β-HSD1 mRNA is often overexpressed in breast cancer tissues[15] and endometriotic lesions.[16] Therefore, the local reduction of estrogen action by inhibiting this enzyme appears to be a promising therapy with less side effects than existing therapies. Proof of concept for the treatment of breast cancer was recently reported in different mouse-models by applying steroidal compounds *intraperitoneally* or *subcutaneously*.[17, 18] However, these compounds do not seem to be appropriate for clinical application.

The type 2 enzyme in the 17β-hydroxysteroid dehydrogenase family is responsible for the oxidation of E2 to E1 using NAD^+ as cofactor and acts as a biological counterpart of 17β-HSD1. Regarding the therapeutic concept, potential inhibitors of 17β-HSD1 should not affect 17β-HSD2. Furthermore, intrinsic estrogenic effects should be avoided, i.e. potential inhibitors should have no or only little affinity to the estrogen receptors α and β (ERα and ERβ).

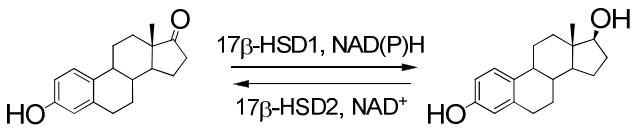
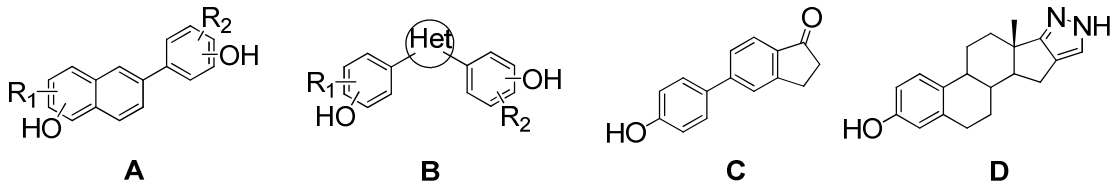


***Chart 1*: Interconversion of estrone (E1) to estradiol (E2)**

Over the last decade, several groups have been working on the development of 17β-HSD1 inhibitors. Most of the latter are based on the steroidal scaffold with expansions at the 6, 15, 16 and 17 positions.[19, 20] Only a few non-steroidal inhibitors have been published so far. In 2006, Messinger et al.[21] described thiophenepyrimidinones as the first non-steroidal inhibitors, which

selectively inhibit 17β-HSD1. Mimicking the E1 skeleton, Allan et al.[22] investigated biphenyl ethanones containing side chains known from the steroidal inhibitors. Recently, we reported on the development of two highly potent and selective, non-steroidal classes of 17β-HSD1 inhibitors, (hydroxyphenyl)naphthols[23-25] (**A**, Chart 2) and bis(hydroxyphenyl)heterocycles[26-31] (**B**, Chart 2). In these investigations molecular modeling studies with X-ray protein structures were performed revealing that all the mentioned inhibitors are likely to bind in the substrate binding site, except the bis(hydroxyphenyl)heterocycles. The latter most probably bind between the cofactor and substrate binding site showing interactions with NADPH.[29]

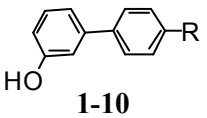


A **B** **C** **D**

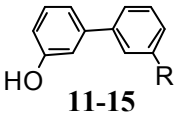
Chart 2: Recently published compound classes from our group: (hydroxyphenyl)naphthols (A), bis(hydroxyphenyl)heterocycles (B) and two known inhibitors (C, D)

In both compound classes recently described by us (**A** and **B**), the most active inhibitors exhibit two OH-groups in a distance of about 11 Å.[23-29] Regarding pharmacokinetics, an exchange of one hydroxyphenyl moiety seems to be desirable because of the susceptibility of phenols to phase II metabolism.[32]

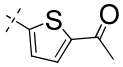
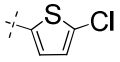
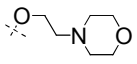
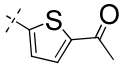
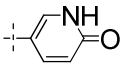
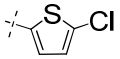
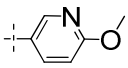
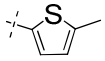
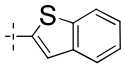
Therefore herein we report on the design of new steroidomimetics bearing only one OH-group. The compounds were designed using a combination of ligand- and structure-based approach. In the following, synthesis and biological evaluation of new non-steroidal 17β-HSD1 inhibitors of the heterocyclic substituted biphenylol type and their aza-analogs are described. Molecular modeling studies were performed in order to elucidate protein-ligand interactions in the active site.

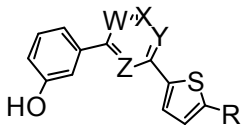


1-10



11-15

cmpd	R	cmpd	R
1		**9**	
2		**10**	
3		**11**	
4		**12**	
5		**13**	
6	H	**14**	
7		**15**	
8			



16-20

cmpd	R	W	X	Y	Z
16	H	CH	CH	N	CH
17	Cl	CH	CH	N	CH
18	Cl	CH	CH	CH	N
19	Cl	N	CH	CH	CH
20	Cl	CH	N	CH	CH

***Chart 3*: Title compounds**

Design

Ligand-based: In order to reduce the risk of undesired side effects, novel compounds should not have affinity to steroid receptors. Accordingly, we focused on the design of non-steroidal inhibitors. In this field we previously designed highly active inhibitors of 17β-HSD1 bearing two OH-groups mimicking the two hydrophilic features of the substrate.[23, 26] Interestingly, some inhibitors containing only one OH-group showed still a notable activity.[27] A prerequisite for their activity is a *meta* OH-phenyl moiety. This substitution pattern will play an important role in the design of the compounds presented in this study.

We herein investigate E1, the natural substrate of 17β-HSD1 as template for ligand-based design, with a *meta* OH-phenyl group mimicking the steroidal A-ring and the replacement of

the D-ring keto function by means of different heterocycles avoiding a second OH-group. The distance between the two hydrophilic features of E1 is approximately 11 Å and will be considered as a further component in the drug design concept. In order to keep the lipophilic character of the steroidal scaffold, the two hydrophilic anchor points will be linked by the non-hydrophilic benzene moiety.

A similar approach was applied by Allan et al.[22] who also investigated E1-mimetics. In their study phenylindanone **C** (Chart 2) bearing a carbonyl function was found to be the most potent inhibitor with an IC_{50} value of 1.7 µM. They also published steroidal compounds bearing a pyrazole E-ring, which showed also remarkable inhibitory activity (compound **D**, IC_{50} = 0.18 µM),[33] indicating that the heterocycle is well tolerated by the enzyme.

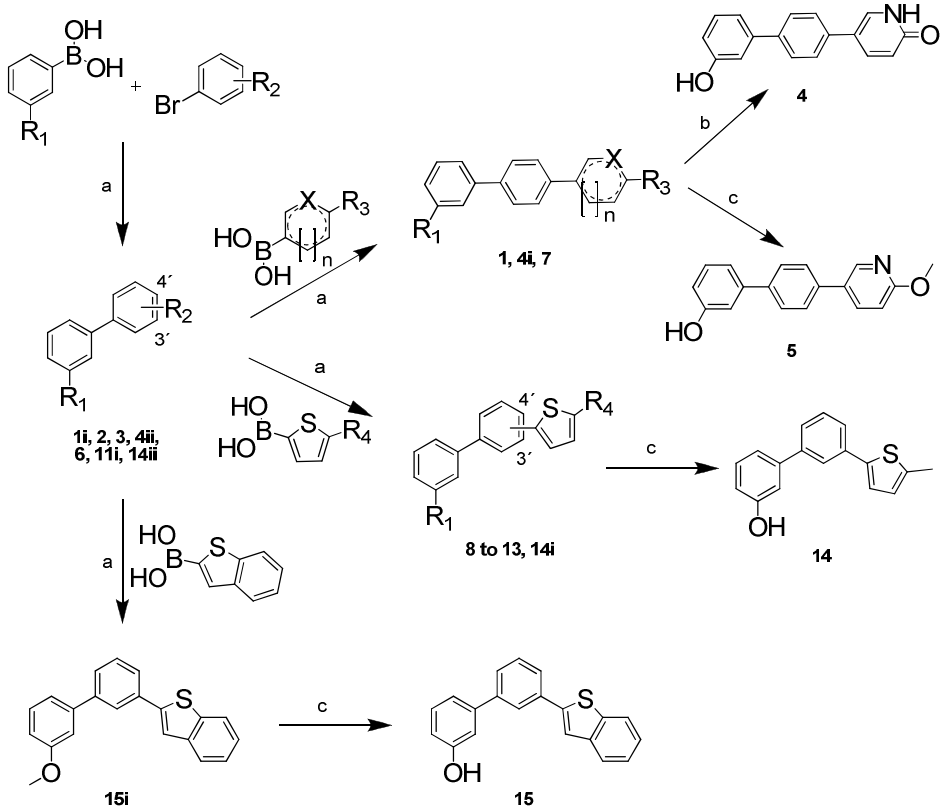
In this study, compound **1** (Chart 3) was chosen as starting point for the development of E1-mimetics as potential 17β-HSD1 inhibitors, with furane replacing the steroidal D-ring. Besides furane, 2-pyridone, morpholine and thiophene seemed to be appropriate moieties to investigate the suitability of further heteroatoms.

Structure-based: Among the numerous published crystal structures[34] there is no structure of the enzyme cocrystallized with E1 but with the closely related, well known 17β-HSD1 inhibitor equilin, an equine estrogen with a C7-C8 double bond and a carbonyl function in position 17. Therefore, the ternary complex of human 17β-HSD1 with equilin and $NADP^+$ (PDB-ID: 1EQU)[35] appeared to be suitable for studying the three-dimensional architecture of the enzyme. A substrate binding site, a cofactor binding pocket and a highly flexible substrate-entry loop (αG´βF) structure can be defined. The steroid binding site is almost exclusively hydrophobic except for two hydrophilic ends: the 17-keto oxygen of equilin accepts protons from Tyr155 and Ser142 at the catalytic end. At the other end, the 3-hydroxy group of equilin establishes a bifurcated hydrogen bond to His221 and Glu282. Closer analysis of the substrate binding pocket reveals three additional polar amino acids (Asn152, Tyr218 and Ser222) in the hydrophobic area, which are not involved in steroid binding. Introduction of heteroatoms in the benzene core of a potential inhibitor might be appropriate to explore supplementary interactions.

Chemistry

The synthesis of benzene derivatives **1-15** is depicted in Scheme 1. Compounds **1-3**, **4i**, **7-13** and **14i-15i** were synthesized via Suzuki reaction starting from the commercially available boronic acids and the brominated benzene derivatives. Microwave assisted Suzuki cross coupling reactions were carried out as one-pot synthesis using method A[27] (Cs_2CO_3, DME/EtOH/water (1:1:1), $Pd(PPh_3)_4$, MW (150 W, 150 °C, 15 bar, 15 min)). The ether

functions of the methylated hydroxyphenyl derivatives **5**, **14** and **15** were cleaved according to method D[27] (BBr_3, CH_2Cl_2, -78 °C to rt, 18 h). The pyridin-2(1*H*)-one derivative **4** was prepared by demethylation of intermediate **4i** with borontrifluoride dimethyl sulfide complex by stirring the reaction mixture at rt for 20 hours in anhydrous dichloromethane.



cmpd	R_1	R_2	R_3	X	n
1i	OH	4´-Br			
1	OH		H	O	0
2	OH	4´-morpholino			
3	OH	4´-(2-morpholinoethoxy)			
4ii	OCH_3	4´-Br			
4i	OCH_3		OCH_3	N	1
6	H	OH			
7	OH		H	S	0

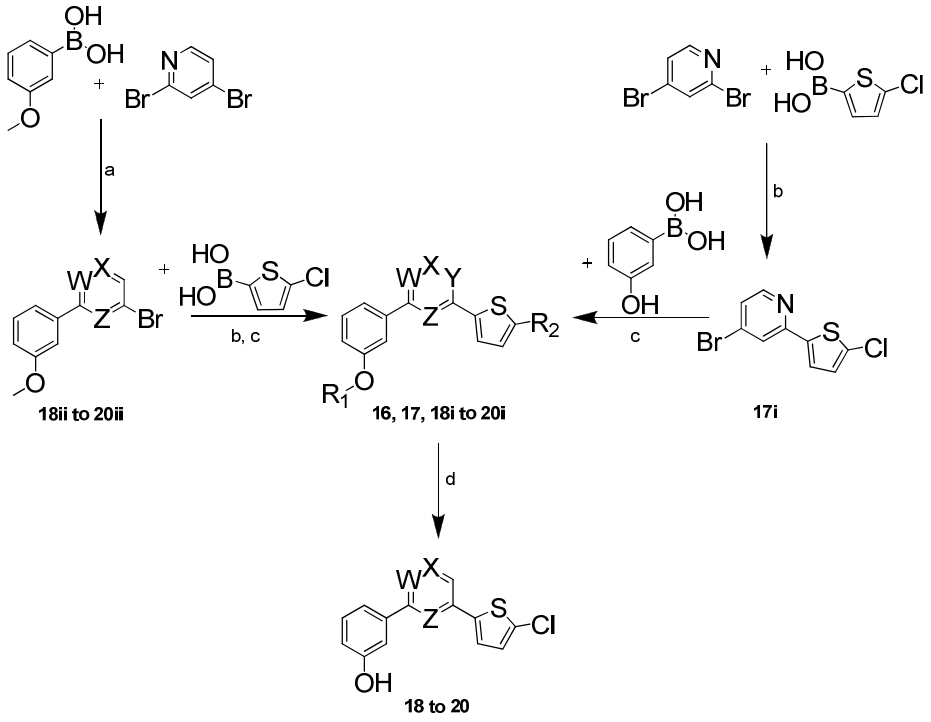
cmpd	R_1	R_2	thienyl	R_4
8	OH		4´	H
9	OH		4´	CO-CH_3
10	OH		4´	Cl
11i	OH	3´-Br		
11	OH		3´	CO-CH_3
12	OH		3´	Cl
13	OH		3´	H
14ii	OCH_3	3´-Br		
14i	OCH_3		3´	CH_3

***Scheme 1*: Synthesis of compounds 1-15**[a]

[a] reagents and conditions: (a) Method A: Cs_2CO_3, $Pd(PPh_3)_4$, DME/EtOH/water (1:1:1), microwave conditions (150 W, 15 bar, 150 °C, 15 min); (b) $BF_3 \cdot SMe_2$, CH_2Cl_2, rt, 20 h; (c) Method D: BBr_3, CH_2Cl_2, -78 °C, 20 h

The synthetic routes for the pyridine derivatives **16-20** are shown in Scheme 2. Starting from the commercially available dibrominated pyridines and boronic acid derivatives, compounds **17i** and **19ii** were obtained via Suzuki reaction following the conditions of method B (K_2CO_3, toluene/EtOH/water (1:1:1), $Pd(PPh_3)_4$, 100 °C, 18 h) and method C ($NaHCO_3$, toluene/water (1:1), $Pd(PPh_3)_4$, 100 °C, 18 h), respectively. Method A was used for the coupling of 3-hydroxybenzene boronic acid with compound **17i** to yield compound **17**. Compound **16** devoid of the chlorine substituent at the thiophene was isolated as a side product.

Cross coupling of 5-chloro-2-thienyl boronic acid with brominated pyridine derivatives **18ii-20ii** according to method A (Cs_2CO_3, DME/EtOH/water (1:1:1), $Pd(PPh_3)_4$, MW (150 W, 150 °C, 15 bar, 15 min)) and method B (K_2CO_3, toluene/EtOH/water (1:1:1), $Pd(PPh_3)_4$, 100 °C, 18 h) led to compounds **19i** (method A), **18i** and **20i** (method B). Compounds **18-20** were prepared by cleaving the methoxy function with boron tribromide (method D: BBr_3, CH_2Cl_2, -78 °C to rt, 18 h).



middle ring = pyridine
16, 17: Y = N
18ii, 18i, 18: Z = N
19ii, 19i, 19: W = N
20ii, 20i, 20: X = N

cmpd	R_1	R_2	method
16	H	H	c
17	H	Cl	c
18i	CH_3	Cl	b
19i	CH_3	Cl	c
20i	CH_3	Cl	b

***Scheme 2*: Synthesis of compounds 16-20**[a]

[a] reagents and conditions: (a) Method C: $NaHCO_3$, $Pd(PPh_3)_4$, toluene/water (1:1), 100°C, 18 h; (b) Method B: K_2CO_3, $Pd(PPh_3)_4$, toluene/EtOH/water (1:1:1), 100°C, 18 h; (c) Method A: Cs_2CO_3, $Pd(PPh_3)_4$, DME/EtOH/water (1:1:1), microwave conditions (150 W, 15 bar, 150 °C, 15 min); (d) Method D: BBr_3, CH_2Cl_2, -78 °C, 20 h

Biological results

Activity: Inhibition of human 17β-HSD1

Placental enzyme isolated following a described procedure[23] was incubated with tritiated E1, cofactor and inhibitor. The separation of substrate and product was performed by HPLC. Compounds showing less than 10 % inhibition at 1 µM were considered to be inactive. The percent inhibition values of compounds **1-20** are shown in Table 1. The IC_{50}-values of selected compounds are depicted in Table 2.

cmpd	% inhibition[a]	
	17β-HSD1[b]	17β-HSD2[c]
1	17	n.i.
2	n.i.	n.i.
3	n.i.	n.i.
4	n.i.	n.i.
5	58	35
6	n.i.	n.i.
7	41	36
8	36	22
9	38	10
10	48	n.i.
11	22	23
12	71	31
13	48	40
14	32	36
15	49	39
16	33	62
17	39	63
18	40	21
19	61	25
20	40	46

Table 1: Inhibition of human 17β-HSD1 and 17β-HSD2 by compounds 1-20

[a] Mean value of three determinations, standard deviation less than 10 %, compound concentration:1 µM
[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 %)

Compound **1**, bearing a furane moiety only showed marginal inhibitory activity of 17 % at a concentration of 1 µM. To get more insight into the role of the heterocycles, we synthesized compounds **2-5**, **7** and **8** differing in the heteroatoms, the electronic and the geometric properties of the heterocycle. Compounds **2-3**, in which furane is replaced with non-aromatic heterocycles, showed no activity, indicating that a lack of aromaticity is counterproductive. The inactivity of pyridone **4** supports this hypothesis since its aromatic character is less pronounced compared to the methoxy pyridine **5**.[36] Pyridine **5** and thiophenes **7**, **8** showing moderate activity (58, 41 and 36 % inhibition, respectively) seemed to be appropriate mimics of the

steroidal keto-function. Regarding compound **5** (IC_{50} = 0.69 µM, Table 2), the methoxy-group and the pyridine nitrogen offer hydrogen bond acceptor properties. This means that both the pyridine-nitrogen and the methoxy oxygen are able to form hydrogen bonds with the catalytic amino acids. The slightly reduced activity of **7** and **8** might be explained by the lack of these hydrogen bond interactions. Introduction of an electron withdrawing (**9**) as well as an electron donating (**10**) substituent did not improve potency significantly.

As the 1,4-disubstituted benzene derivatives **9** and **10** are larger than 11 Å, the 1,3-disubstituted **11** and **12** were synthesized in order to readjust the E1-derived requirements. While the activity of the acetylthiophene derivative **11** decreased, the chlorine substituted thiophene **12** is the most potent inhibitor of 17β-HSD1 identified in this study with an IC_{50} of 0.56 µM. Compounds **13** and **14** bearing H and CH_3 instead of Cl and compound **15** with benzothiophene substituent, were synthesized as tools to evaluate the impact of the chlorine atom on the activity and get insight into possible inhibitor-protein interactions. They all turned out to be less active than **12**, highlighting the positive effect of chlorine for enzyme affinity.

As discussed above, insertion of a heteroatom, capable of acting as hydrogen bond acceptor, in the central benzene core might lead to additional interactions with Tyr218 and Ser222. For the sake of clarifying this hypothesis, the benzene core was replaced by pyridine, leading to isomeric compounds **16-20**. None of the compounds in this series enhances 17β-HSD1 inhibition, indicating that the pyridine nitrogen is not able to target these hydrophilic amino acids. In case of compounds **17**, **18** and **20**, the insertion of a nitrogen even decreases inhibitory activity compared to **12**. Nevertheless, the isomer **19** with an IC_{50}-value of 0.85 µM showed nearly the same activity as compound **12**.

Selectivity: Inhibition of human 17β-HSD2 and affinities for ERα and ERβ

Since 17β-HSD2 catalyzes the oxidative transformation of E2 into E1, inhibitory activity toward this enzyme must be avoided. 17β-HSD2 inhibition was determined using an assay similar to the 17β-HSD1 test. Placental microsomes were incubated with tritiated E2 in the presence of NAD^+ and inhibitor. Quantification of labelled product formed was performed by HPLC and subsequent radio detection. Compounds showing less than 10 % inhibition at 1 µM were considered to be inactive. Percentage of inhibition and IC_{50}-values are shown in Tables 1 and 2. All compounds, which did not inhibit 17β-HSD1 were also inactive on the type 2 enzyme.

Comparing the isomeric pyridines **17** and **19**, it is striking that the position of the nitrogen has a major impact on the selectivity. Compound **17**, bearing the pyridine nitrogen next to the thiophene, showed an IC_{50} of 0.47 µM for 17β-HSD2, which demonstrates a 5 fold higher inhibition than for the type 1 enzyme. Having the position of the nitrogen next to the hydroxyphenyl ring (**19**), the selectivity changed completely (4-fold higher affinity toward 17β-

HSD1). According to the position of the nitrogen, a 17β-HSD1 inhibitor can be transformed into a 17β-HSD2 inhibitor. Among the compounds revealing 17β-HSD1 activity, however, the chlorothiophene **10** showed the best selectivity (> 10 fold) toward 17β-HSD2.

The therapeutic concept of 17β-HSD1 inhibition includes that potential inhibitors should have no or low affinity toward ERα and ERβ in order to avoid systemic effects. Binding affinities for ERα and ERβ were measured for selected compounds and are shown in Table 2. Using recombinant human protein, a competition assay applying tritium labelled E2 (RBA=100 %) was performed. All evaluated compounds present very low binding affinity to both estrogen receptors.

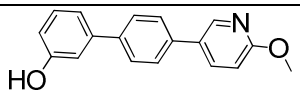
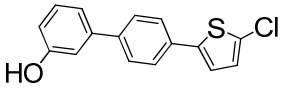
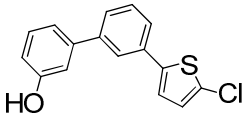
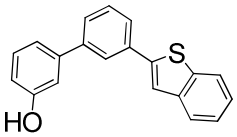
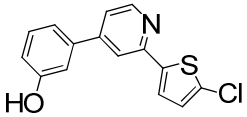
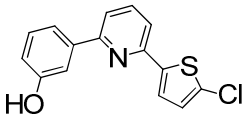
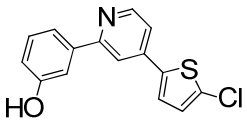
cmpd	structure	IC_{50}[a] (µM)		RBA[d] (%)	
		17β-HSD1[b]	17β-HSD2[c]	ERα	ERβ
5		0.69	2.97	0.001<x<0.01	<0.001
10		1.02	>10	0.01<x<0.1	0.001<x<0.01
12		0.56	2.37	<0.01	<0.001
15		1.37	1.94	<0.01	<0.001
17		2.38	0.47	0.001<x<0.01	<0.001
18		1.39	7.11	0.001<x<0.01	<0.001
19		0.85	3.64	<0.001	<0.001

***Table 2*: Inhibition of 17β-HSD1, 17β-HSD2 and binding affinities for the estrogen receptors α and β by selected compounds**

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

Molecular Modeling

In order to gain deeper insight into plausible molecular interactions between the synthesized steroidomimetics and 17β-HSD1, docking experiments were performed using the monomer of the X-ray structure 1EQU (PDB-ID).

Selected compounds were docked into 17β-HSD1 by means of the docking software Autodock4.2[37] and GOLD4.0.1.[38] In Figure 1A, a plausible binding pose for **5** is shown as an example. The compound is found in the steroidal binding mode. The *meta* OH-group makes hydrogen bonds with His221 and Glu282 ($d_{O\text{-}N}$ = 3.2 Å and $d_{O\text{-}O}$ = 2.7 Å) in a bifurcated fashion, mimicking the OH-group of the steroidal A-ring. The methoxy substituted pyridine ring points toward the catalytic region of the protein. The pyridine nitrogen and the methoxy oxygen might be implicated in hydrogen bonds with Ser142 ($d_{O\text{-}N}$ = 4.0 Å) and Tyr155 ($d_{O\text{-}O}$ = 3.8 Å), respectively. The docking studies also showed that the equipotent **10** binds in the same area as compound **5** (Figure 1), comparable to equilin cocristallized with 17β-HSD1 (1EQU). It is not clear if the interactions established by the methoxypyridine are relevant for the stabilisation of **5** as the chlorothiophene moiety of **10** is located in the same area and is not able to establish the same H-bond interaction patterns.

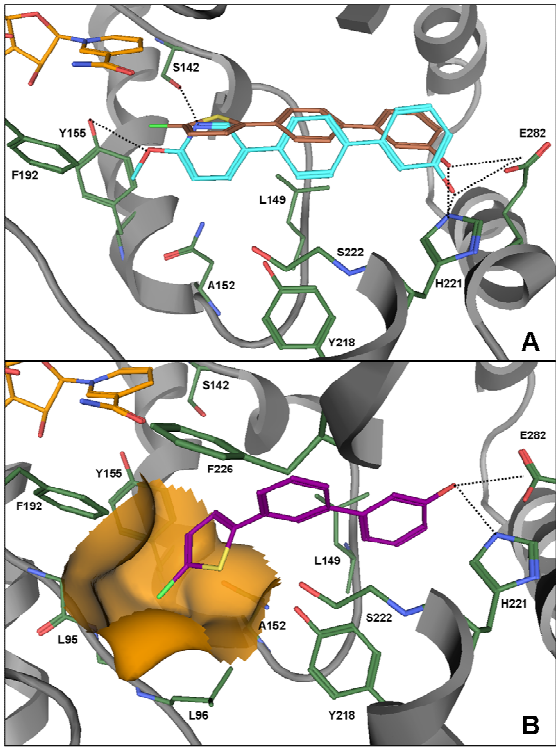


***Figure 1**: Docking complex between 17β-HSD1 (PDB-ID: 1EQU) and compounds 5 (cyan), 10 (brown) overlay depicted in Figure 1A and 12 (violet) depicted in Figure 1B. Cofactor NADPH (orange), interacting residues (green) and cartoon rendered tertiary structure (grey) of the active site are shown. Hydrogen bonds are drawn in black dashed lines. The surface of the subpocket is illustrated (light brown) in Figure 1B. Figures generated with MOE (Chemical Computing Group Inc., Montreal, Canada).*

Taking in account that compound **12** was designed to better fit the E1-distance requirements, the same interactions as with the linear molecules were expected for this compound.
However, the results of the docking experiments indicate that the chlorothiophene moiety of **12** is not directed toward the catalytic center (Figure 1B). While the hydroxyphenyl group is still imitating the steroidal A-ring ($d_{O\text{-}N}$ = 2.7 Å and $d_{O\text{-}O}$ = 3.5 Å), the non-linear scaffold enables the heterocycle to point to a rather lipophilic subpocket consisting of Leu95, Leu96, Asn152, Tyr155 and Phe192. Compounds **13**-**15** were found in the same binding mode as **12** (data not shown).

Discussion and Conclusions

The biological results described herein provide evidence that mimicking the chemical features of E1 is an appropriate approach for the development of new 17β-HSD1 inhibitors. While in previous studies, the inhibitors bore two OH-groups, here only one OH-group was retained, namely the *meta* OH-phenyl group imitating the A-ring. The study was mainly focused on the investigation of (substituted) heterocycles as suitable D-ring mimics. It is shown that compounds **2** and **3**, with an aliphatic heterocycle replacing the D-ring, showed no inhibitory activity. This indicates that flatness and a delocalised π-system are prerequisites for inhibitory activity.
The interaction pattern of Ser142, Tyr155 and the 17β-OH group of E2 has been described to be a triangle-shaped hydrogen bond network.[39] Until now, it was not clarified whether the hydrogen bond donor function is a crucial component of this network. In this study we were able to show that heterocycles like methoxypyridine and chlorothiophene are able to unfold high inhibitory activity.
The results of the docking study showed that the 1,3-benzene derivatives most likely do not exclusively cover the substrate binding area. Parts of them were found to reach into the rather lipophilic subpocket recently described by Mazumdar et al.[40] As the chlorothiophene moiety of compound **12** was found to bind into this subpocket, compounds **13**-**15** were designed to validate this finding. Since the benzothiophene moiety of **15** is too bulky to fit into the area of the catalytic center as observed for compounds **5** and **10**, the only slightly reduced inhibitory activity of **15** compared to **12** supports our hypothesis of these ligands binding to the subpocket. The high to moderate inhibitory activity of compounds **12** and **15** can be explained by hydrophobic and van der Waals interactions within this subpocket. In order to investigate the relevance of the chloro substituent for the activity, compounds **13** and **14** were synthesized replacing Cl by H (**13**) and by CH_3 (**14**). Compound **13** is a weaker inhibitor of 17β-HSD1 compared to the parent compound (**12**), which might be due to its decreased lipophilicity. However, compound **14** shows less inhibition compared to **12**, although chloro and methyl

exhibit very similar lipophilic properties. This indicates that not only lipophilicity is responsible for high affinity to this subpocket. In this context, the different electronic characteristics of the substituents as well as the possible formation of Cl-π interactions[41] might be involved in the increased potency of **12**.

With the aim of increasing activity and selectivity the middle benzene ring was exchanged by a pyridine in order to target the hydrophilic amino acids Tyr218 and Ser222 in the predominantly hydrophobic binding site. According to the expected binding mode, the distances between the pyridine nitrogens of compounds **16-20** and these residues should be in an appropriate range for hydrogen bonding. However, none of these aza-analogs enhanced 17β-HSD1 inhibitory activity compared to **12**. The nitrogens are obviously neither able to interact with these amino acids nor can any additional interaction of the nitrogens be observed. The biological data might be explained by differences in molecular electrostatic potential (MEP) distributions which were found to play an important role in the inhibition of 17β-HSD1 by other compounds, too.[27, 29] For these it was shown that insertion of nitrogen and/or exchange of its position have a high impact on the MEP distributions.[27]

The selectivity of compounds **17-20** is influenced by the position of the nitrogen. As there is neither a crystal structure nor an appropriate homology model of 17β-HSD2 available, protein-ligand interactions cannot be interpreted. Comparing the aza-compounds it becomes apparent that only compound **17** showed a significantly higher inhibitory activity toward 17β-HSD2. In this compound, the nitrogen close to the chlorothiophene ring is responsible for a more negative electrostatic potential in this part of the structure (see MEP calculations in supporting material). This distribution might be beneficial for 17β-HSD2 inhibition whereas a negative MEP in the area of the *meta* hydroxyphenyl ring led to higher inhibitory activity toward 17β-HSD1.

In summary, a new series of heterocyclic substituted biphenylols and their aza-analogs was designed as 17β-HSD1 inhibitors and synthesized by combining a ligand- and structure-based approach. Among these, a promising compound was discovered (**12**), which showed high activity, selectivity over 17β-HSD2 and very low affinity toward the ERs. The binding mode of the angulate 1,3-benzene derivatives differed from that observed for the linear compounds. The latter superimpose well to equilin which indicates that an OH-group pointing into the catalytic center is not necessary for high inhibitory activity and can be taken over by other subtituents like methoxypyridine or chlorothiophene. This observation might be important for the design of other classes of 17β-HSD1 inhibitors. Furthermore it seems feasible that by combination of the two interaction patterns observed in this study (angulate-shaped structures interacting with the subpocket and linear structures with the catalytic center) a further increase of activity and selectivity could be achieved leading to the development of clinical candidates.

Experimental section

Chemical methods

Chemical names follow IUPAC nomenclature. Starting materials were purchased from Aldrich, Acros, Lancaster, Roth, Combi Blocks, Merck or Fluka and were used without purification.
Column chromatography (CC) was performed on silica gel (70-200 μm) coated with silica, preparative thin layer chromatography (TLC) on 1 mm SIL G-100 UV_{254} glass plates (Macherey-Nagel) and reaction progress was monitored by TLC on Alugram SIL G UV_{254} (Macherey-Nagel).
IR spectra were recorded on a Bruker Vector 33 spectrometer (neat sample).
^{1}H-NMR and ^{13}C-NMR spectra were measured on a Bruker AM500 spectrometer (500 MHz) at 300 K. Chemical shifts are reported in δ (parts per million: ppm), by reference to the hydrogenated residues of deuteriated solvent as internal standard ($CDCl_3$: δ = 7.24 ppm (^{1}H-NMR) and δ = 77 ppm (^{13}C-NMR), CD_3OD: δ = 3.35 ppm (^{1}H-NMR) and δ = 49.3 ppm (^{13}C-NMR), CD_3COCD_3: δ = 2.05 ppm (^{1}H-NMR) and δ = 29.9 ppm (^{13}C-NMR), CD_3SOCD_3 δ = 2.50 ppm (^{1}H-NMR) and δ = 39.5 ppm (^{13}C-NMR)). Signals are described as s, d, t, dd, ddd, m, dt, q for singlet, doublet, triplet, doublet of doublets, doublet of doublets of doublets, multiplet, doublet of triplets and quadruplet respectively. All coupling constants (*J*) are given in hertz (Hz).
Mass spectra (ESI and APCI) were recorded on a TSQ Quantum (Thermo Finnigan) instrument.
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: 4'-bromobiphenyl-3-ol (**1i**),[42] 4'-bromo-3-methoxybiphenyl (**4ii**),[43] biphenyl-3-ol (**6**),[44] 3'-bromobiphenyl-3-ol (**11i**),[45] 3-bromo-3'-methoxybiphenyl (**14ii**),[46] 2-bromo-6-(3-methoxyphenyl)pyridine (**18ii**),[47] 3-bromo-5-(3-methoxyphenyl)pyridine (**20ii**)[48].

General procedure for Suzuki coupling

Method A: A mixture of arylbromide (1 eq), boronic acid derivative (1.2 eq), caesium carbonate (4 eq) and tetrakis(triphenylphosphine) palladium (0.03 eq) was suspended in an oxygen free DME/EtOH/water (1:1:1) mixture. The reaction mixture was exposed to microwave irradiation (15 min, 150 W, 150 °C, 15 bar). After cooling to rt, water was added and the aqueous layer was extracted with ethyl acetate. The combined organic layers were washed with brine, dried over sodium sulfate, filtered and concentrated to dryness. The product was purified by CC, preparative TLC or preparative HPLC, respectively.

Method B: A mixture of arylbromide (1 eq), boronic acid derivative (1.2 eq), potassium carbonate (2 eq) and tetrakis(triphenylphosphine) palladium (0.03 eq) in an oxygen-free toluene/EtOH/water (1:1:1) mixture was stirred at 100 °C for 18 h under nitrogen atmosphere. The reaction mixture was cooled to rt. The aqueous layer was extracted with ethyl acetate. The combined organic layers were washed with brine, dried over sodium sulfate, filtered and concentrated to dryness. The product was purified by CC.

Method C: A mixture of aryldibromide (1 eq), methoxybenzene boronic acid (1 eq), sodium hydrogencarbonate (2 eq) and tetrakis(triphenylphosphine) palladium (0.03 eq) in an oxygen free toluene/water (1:1) mixture was stirred at 100 °C for 18 h under nitrogen atmosphere. The reaction mixture was cooled to rt. The aqueous layer was extracted with ethyl acetate. The combined organic layers were washed with brine, dried over magnesium sulfate, filtered and concentrated in vacuo. The product was purified by CC.

General procedure for ether cleavage

Method D: To a solution of methoxybenzene derivative (1 eq) in anhydrous dichloromethane at -78 °C (dry ice/acetone bath), boron tribromide in dichloromethane (1 M, 3 eq) was added dropwise. The reaction mixture was stirred for 20 h to rt 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 sodium sulfate, evaporated to dryness under reduced pressure and purified by CC, preparative TLC or preparative HPLC, respectively.

General procedure for purification using preparative HPLC

All declared compounds were purified via an Agilent Technologies Series 1200–preparative HPLC using a linear gradient run (solvents: acetonitrile, water) starting from 20 % acetonitrile up to 100 % in 36 min.

Detailed synthesis procedure for all compounds

4'-(3-Furyl)biphenyl-3-ol (**1**). The title compound was prepared by reaction of 4'-bromobiphenyl-3-ol (**1i**) (150 mg, 0.60 mmol), furane-3-boronic acid (81 mg, 0.72 mmol), caesium carbonate (785 mg, 2.40 mmol) and tetrakis(triphenylphosphine) palladium (21 mg, 18 μmol) according to method A. The product was purified by preparative TLC (hexane/ethyl acetate 7:3); yield: 3 % (4 mg); MS (ESI): 237 $(M+H)^+$; ^{1}H NMR (CD_3COCD_3): 8.44 (s, 1H), 8.06 (dd, J = 0.9 Hz and J = 1.6 Hz, 1H), 7.69-7.67 (m, 2H), 7.65-7.63 (m, 3H), 7.28 (t, J = 8.1 Hz, 1H), 7.15-7.13 (m, 2H), 6.92 (dd, J = 0.9 Hz and J = 1.9 Hz, 1H), 6.84 (ddd, J = 0.9 Hz and

J = 2.5 Hz, and *J* = 8.2 Hz, 1H); ^{13}C NMR (CD_3COCD_3): 162.50, 146.10, 144.00, 141.10, 133.50, 131.80, 129.10, 128.10, 128.00, 119.90, 118.30, 118.10, 116.30, 115.50, 110.50; IR: 3452, 3413, 3281, 1597, 1164, 835, 780 cm^{-1}.

4'-Morpholin-4-ylbiphenyl-3-ol (**2**). The title compound was prepared by reaction of 4-(4-bromophenyl)morpholine (150 mg, 0.62 mmol), 3-hydroxybenzene boronic acid (103 mg, 0.74 mmol), caesium carbonate (807 mg, 2.48 mmol) and tetrakis(triphenylphosphine) palladium (22 mg, 19 μmol) according to method A. The product was crystallized in ethyl acetate; yield: 27 % (42 mg); MS (ESI): 256 $(M+H)^+$; ^{1}H NMR (CD_3SOCD_3): 7.01 (d, *J* = 8.8 Hz, 2H), 6.73 (t, *J* = 7.9 Hz, 1H), 6.55-6.53 (m, 3H), 6.50 (t, *J* = 2.2 Hz, 1H), 6.22 (ddd, *J* = 0.9 Hz and *J* = 2.2 Hz and *J* = 7.9 Hz, 1H), 3.29 (t, *J* = 4.7 Hz, 4H), 2.69 (t, *J* = 4.7 Hz, 4H); ^{13}C NMR (CD_3SOCD_3): 149.10, 132.70, 122.20, 121.10, 118.40, 108.00, 106.60, 104.80, 104.00, 57.40, 39.90; IR: 3245, 2957, 1585, 1207, 1110, 828, 785 cm^{-1}.

4'-(2-Morpholin-4-ylethoxy)biphenyl-3-ol (**3**). The title compound was prepared by reaction of 4-[2-(4-bromophenoxy)ethyl]morpholine (150 mg, 0.52 mmol), 3-hydroxybenzene boronic acid (87 mg, 0.63 mmol), caesium carbonate (678 mg, 2.08 mmol) and tetrakis(triphenylphosphine) palladium (18 mg, 16 μmol) according to method A. The product was purified by CC (dichloromethane/methanol 96:4) followed by preparative HPLC; yield: 20 % (32 mg); MS (ESI): 300 $(M+H)^+$; ^{1}H NMR (CD_3COCD_3): 8.19 (s, 1H), 7.54 (d, *J* = 8.8 Hz, 2H), 7.23 (t, *J* = 7.6 Hz, 1H), 7.07-7.05 (m, 2H), 7.00 (d, *J* = 8.8 Hz, 2H), 6.78 (ddd, *J* = 1.3 Hz and *J* = 2.5 Hz and *J* = 8.2 Hz, 1H), 4.18 (t, *J* = 5.7 Hz, 2H), 3.64 (t, *J* = 4.7 Hz, 4H), 2.82 (t, *J* = 5.7 Hz, 2H), 2.60 (t, *J* = 4.7 Hz, 4H); ^{13}C NMR (CD_3COCD_3): 164.50, 160.40, 159.70, 144.00, 135.40, 131.70, 129.70, 119.50, 116.70, 115.60, 115.20, 68.20, 67.60, 59.20, 55.80; IR: 3405, 2868, 1607, 1477, 1116, 835, 783 cm^{-1}.

2-Methoxy-5-(3'-methoxybiphenyl-4-yl)pyridine (**4i**). The title compound was prepared by reaction of 4'-bromo-3-methoxybiphenyl (**4ii**) (300 mg, 1.14 mmol), 2-methoxy-5-pyridineboronic acid (209 mg, 1.37 mmol), caesium carbonate (1486 mg, 4.56 mmol) and tetrakis(triphenylphosphine) palladium (40 mg, 34 μmol) according to method A. The product was purified by CC (hexane/ethyl acetate 9:1); yield: 84 % (280 mg); ^{1}H NMR ($CDCl_3$): 8.45 (d, *J* = 2.2 Hz, 1H), 7.83 (dd, *J* = 2.5 Hz and *J* = 8.5 Hz, 1H), 7.67 (d, *J* = 8.5 Hz, 2H), 7.60 (d, *J* = 8.5 Hz, 2H), 7.38 (t, *J* = 7.9 Hz, 1H), 7.23-7.22 (m, 1H), 7.17 (t, *J* = 2.1 Hz, 1H), 6.93-6.91 (m, 1H), 6.84 (dd, *J* = 0.6 Hz and *J* = 8.5 Hz, 1H), 4.00 (s, 3H), 3.88 (s, 3H); ^{13}C NMR ($CDCl_3$): 163.70, 160.00, 144.90, 142.10, 140.10, 137.30, 137.00, 129.90, 129.60, 127.70, 127.00, 119.50, 112.80, 110.90, 55.30, 53.50.

5-(3'-Hydroxybiphenyl-4-yl)pyridin-2(1*H*)-one (**4**). To a solution of 2-methoxy-5-(3'-methoxybiphenyl-4-yl)pyridine (**4i**) (150 mg, 0.52 mmol, 1 eq) in dry dichloromethane, borontrifluoride dimethyl sulfide complex (39 mmol, 75 eq) was added dropwise at rt. The reaction mixture was stirred for 20 h at rt. Water was added to quench the reaction and the aqueous layer was extracted with ethyl acetate. The combined organic layers were washed with brine, dried over sodium sulfate, evaporated to dryness under reduced pressure and purified by preparative TLC (hexane/ethyl acetate 1:1); yield: 12 % (17 mg); MS (ESI): 264 $(M+H)^+$; ^{1}H NMR (CD_3SOCD_3): 11.82 (s, 1H), 9.51 (s, 1H), 7.87 (dd, *J* = 2.9 Hz and *J* = 9.5 Hz, 1H), 7.75 (d, *J* = 2.9 Hz, 1H), 7.62 (s, 4H), 7.25 (t, *J* = 7.9 Hz, 1H), 7.10-7.08 (m, 1H), 7.04 (t, *J* = 2.1 Hz, 1H), 7.76 (ddd, *J* = 0.9 Hz and *J* = 2.5 Hz and *J* = 8.2 Hz, 1H), 6.45 (d, *J* = 9.5 Hz, 1H); ^{13}C NMR (CD_3SOCD_3): 161.70, 157.80, 140.90, 139.80, 138.40, 135.10, 132.60, 129.90, 127.20, 126.90, 125.50, 122.80, 120.00, 117.20, 114.40, 113.20; IR: 3134, 2921, 1657, 1584, 1205, 825, 779 cm^{-1}.

4'-(6-Methoxypyridin-3-yl)biphenyl-3-ol (**5**). The title compound was prepared by reaction of 2-methoxy-5-(3'-methoxybiphenyl-4-yl)pyridine (**4i**) (30 mg, 0.10 mmol) and boron tribromide (0.30 mmol) according to method D. The product was purified by preparative TLC (hexane/ethyl acetate 1:1); yield: 55 % (16 mg); MS (ESI): 292 $(M+H)^+$; ^{1}H NMR (CD_3COCD_3): 8.49-8.47 (m, 2H), 7.99 (dd, *J* = 2.9 Hz and *J* = 8.9 Hz, 1H), 7.71 (s, 4H), 7.29 (t, *J* = 8.1 Hz, 1H), 7.17-7.16 (m, 2H), 6.87-6.85 (m, 2H), 3.94 (s, 3H); ^{13}C NMR (CD_3COCD_3): 165.60, 159.90, 146.70, 143.80, 141.90, 139.20, 138.60, 131.90, 131.30, 129.30, 128.70, 119.90, 116.40, 115.50, 112.60, 54.70; IR: 3234, 3023, 2955, 1601, 1487, 1298, 822 cm^{-1}.

4'-(3-Thienyl)biphenyl-3-ol (**7**). The title compound was prepared by reaction of 4'-bromobiphenyl-3-ol (**1i**) (150 mg, 0.60 mmol), thiophene-3-boronic acid (93 mg, 0.72 mmol), caesium carbonate (785 mg, 2.40 mmol) and tetrakis(triphenylphosphine) palladium (21 mg, 18 µmol) according to method A. The product was purified by preparative TLC (hexane/ethyl acetate 7:3); yield: 19 % (28 mg); MS (ESI): 253 $(M+H)^+$; ^{1}H NMR (CD_3COCD_3): 8.44 (s, 1H), 7.79-7.77 (m, 3H), 7.67 (d, *J* = 8.5 Hz, 2H), 7.57 (d, *J* = 2.2 Hz, 2H), 7.28 (t, *J* = 8.2 Hz, 1H), 7.17-7.15 (m, 2H), 6.85 (ddd, *J* = 1.3 Hz and *J* = 2.2 Hz and *J* = 7.9 Hz, 1H); ^{13}C NMR (CD_3COCD_3): 192.70, 159.80, 143.90, 143.60, 141.60, 136.90, 131.90, 129.20, 128.60, 128.50, 128.00, 122.40, 119.90, 116.30, 115.50; IR: 3479, 3394, 3096, 1596, 1200, 835, 775 cm^{-1}.

4'-(2-Thienyl)biphenyl-3-ol (**8**). The title compound was prepared by reaction of 4'-bromobiphenyl-3-ol (**1i**) (150 mg, 0.60 mmol), thiophene-2-boronic acid (93 mg, 0.72 mmol), caesium carbonate (785 mg, 2.40 mmol) and tetrakis(triphenylphosphine) palladium (21 mg, 18 µmol) according to method A. The product was purified by preparative TLC (hexane/ethyl

acetate 7:3); yield: 22 % (34 mg); MS (APCI): 251 $(M-H)^+$; ^{1}H NMR (CD_3COCD_3): 8.47 (s, 1H), 7.74 (d, *J* = 8.6 Hz, 2H), 7.66 (d, *J* = 8.6 Hz, 2H), 7.49 (d, *J* = 3.8 Hz, 1H), 7.45 (d, *J* = 3.8 Hz, 1H), 7.29 (t, *J* = 8.1 Hz, 1H), 7.16-7.12 (m, 3H), 6.87-6.85 (m, 1H); ^{13}C NMR (CD_3COCD_3): 159.90, 145.60, 143.60, 142.00, 135.40, 131.90, 130.20, 129.30, 127.90, 127.00, 125.30, 119.80, 116.40, 115.40; IR: 3504, 3392, 1596, 1447, 1166, 821 cm^{-1}.

1-[5-(3'-Hydroxybiphenyl-4-yl)-2-thienyl]ethanone (**9**). The title compound was prepared by reaction of 4'-bromobiphenyl-3-ol (**1i**) (100 mg, 0.40 mmol), 5-acetylthiophene-2-boronic acid (82 mg, 0.48 mmol), caesium carbonate (521 mg, 1.60 mmol) and tetrakis(triphenylphosphine) palladium (14 mg, 12 µmol) according to method A. The product was purified by CC (dichloromethane/methanol 98:2) followed by preparative HPLC; yield: 5 % (6 mg); MS (APCI): 295 $(M+H)^+$; ^{1}H NMR (CD_3COCD_3): 8.47 (s, 1H), 7.87 (d, *J* = 4.1 Hz, 1H), 7.84 (d, *J* = 8.5 Hz, 2H), 7.72 (d, *J* = 8.5 Hz, 2H), 7.60 (d, *J* = 4.1 Hz, 1H), 7.30 (t, *J* = 8.1 Hz, 1H), 7.19-7.16 (m, 2H), 6.87 (ddd, *J* = 0.9 Hz and *J* = 2.5 Hz and *J* = 7.9 Hz, 1H), 2.55 (s, 3H); ^{13}C NMR (CD_3COCD_3): 191.70, 153.40, 145.30, 136.10, 132.00, 129.50, 128.40, 126.40, 119.90, 119.30, 115.50, 27.50; IR: 3331, 1634, 1599, 1451, 804 cm^{-1}.

4'-(5-Chloro-2-thienyl)biphenyl-3-ol (**10**). The title compound was prepared by reaction of 4'-bromobiphenyl-3-ol (**1i**) (100 mg, 0.40 mmol), 5-chlorothiophene-2-boronic acid (78 mg, 0.48 mmol), caesium carbonate (521 mg, 1.60 mmol) and tetrakis(triphenylphosphine) palladium (14 mg, 12 µmol) according to method A. The product was purified by CC (dichloromethane) followed by preparative HPLC; yield: 18 % (21 mg); MS (ESI): 287 $(M+H)^+$; ^{1}H NMR (CD_3COCD_3): 8.46 (s, 1H), 7.67 (s, 4H), 7.35 (d, *J* = 4.1 Hz, 1H), 7.29 (t, *J* = 8.1 Hz, 1H), 7.15-7.14 (m, 2H), 7.06 (d, *J* = 3.8 Hz, 1H), 6.86 (ddd, *J* = 0.9 Hz and *J* = 2.2 Hz and *J* = 7.9 Hz, 1H); ^{13}C NMR (CD_3COCD_3): 159.90, 144.60, 143.40, 142.50, 134.40, 131.90, 130.10, 129.80, 129.40, 127.60, 124.90, 119.90, 116.60, 115.50; IR: 3484, 3412, 1584, 1436, 1186, 779 cm^{-1}.

1-[5-(3'-Hydroxybiphenyl-3-yl)-2-thienyl]ethanone (**11**). The title compound was prepared by reaction of 3'-bromobiphenyl-3-ol (**11i**) (150 mg, 0.60 mmol), 5-acetylthiophene-2-boronic acid (123 mg, 0.72 mmol), caesium carbonate (785 mg, 2.40 mmol) and tetrakis(triphenylphosphine) palladium (21 mg, 18 µmol) according to method A. The product was purified by CC (dichloromethane/methanol 98:2) followed by preparative HPLC; yield: 27 % (48 mg); MS (ESI): 295 $(M+H)^+$; ^{1}H NMR (CD_3COCD_3): 8.49 (s, 1H), 7.95 (t, *J* = 1.9 Hz, 1H), 7.84 (d, *J* = 4.1 Hz, 1H), 7.71-7.70 (m, 1H), 7.64-7.62 (m, 2H), 7.52 (t, *J* = 7.6 Hz, 1H), 7.31 (t, *J* = 8.1 Hz, 1H), 7.19-7.17 (m, 2H), 6.89 (ddd, *J* = 0.9 Hz and *J* = 2.5 Hz and *J* = 8.2 Hz, 1H), 2.54 (s, 3H); ^{13}C NMR (CD_3COCD_3): 191.80, 159.80, 153.60, 145.40, 144.00, 143.60,

136.00, 135.80, 131.90, 131.60, 129.40, 127.00, 126.70, 126.40, 120.20, 116.70, 115.80, 27.50; IR: 3365, 3088, 1599, 1443, 1277, 780 cm^{-1}.

3'-(5-Chloro-2-thienyl)biphenyl-3-ol (**12**). The title compound was prepared by reaction of 3'-bromobiphenyl-3-ol (**11i**) (100 mg, 0.40 mmol), 5-chlorothiophene-2-boronic acid (78 mg, 0.48 mmol), caesium carbonate (521 mg, 1.60 mmol) and tetrakis(triphenylphosphine) palladium (14 mg, 12 μmol) according to method A. The product was purified by CC (dichloromethane) followed by preparative HPLC; yield: 9 % (10 mg); MS (APCI): 287 $(M+H)^+$; ^1H NMR (CD_3COCD_3): 8.50 (s, 1H), 7.82-7.81 (m, 1H), 7.58-7.56 (m, 2H), 7.49 (t, *J* = 7.8 Hz, 1H), 7.42 (d, *J* = 3.8 Hz, 1H), 7.30 (t, *J* = 8.2 Hz, 1H), 7.17-7.15 (m, 2H), 7.07 (d, *J* = 3.8 Hz, 1H), 7.89-7.86 (m, 1H); ^{13}C NMR (CD_3COCD_3): 159.90, 144.80, 144.00, 143.80, 135.90, 131.90, 131.60, 130.30, 129.80, 128.50, 126.30, 126.20, 125.70, 125.30, 120.20, 116.60, 115.80; IR: 3535, 3403, 1590, 1188, 778 cm^{-1}.

3'-(2-Thienyl)biphenyl-3-ol (**13**). The title compound was prepared by reaction of 3'-bromobiphenyl-3-ol (**11i**) (100 mg, 0.40 mmol), thiophene-2-boronic acid (62 mg, 0.48 mmol), caesium carbonate (521 mg, 1.60 mmol) and tetrakis(triphenylphosphine) palladium (14 mg, 12 μmol) according to method A. The product was purified by preparative TLC (hexane/ethyl acetate 7:3) followed by preparative HPLC; yield: 36 % (36 mg); MS (ESI): 253 $(M+H)^+$; ^1H NMR (CD_3COCD_3): 8.49 (s, 1H), 7.88 (t, *J* = 1.9 Hz, 1H), 7.66-7.64 (m, 1H), 7.56-7.54 (m, 2H), 7.50-7.47 (m, 2H), 7.30 (t, *J* = 7.9 Hz, 1H), 7.18-7.17 (m, 2H), 7.16-7.14 (m, 1H), 6.88 (ddd, *J* = 0.9 Hz and *J* = 2.2 Hz and *J* = 7.9 Hz, 1H); ^{13}C NMR (CD_3COCD_3): 159.90, 145.80, 144.00, 136.80, 131.90, 131.50, 130.20, 128.00, 127.10, 126.60, 126.00, 125.60, 120.10, 116.50, 115.80; IR: 3365, 1593, 1456, 1186, 777, 692 cm^{-1}.

2-(3'-Methoxybiphenyl-3-yl)-5-methylthiophene (**14i**). The title compound was prepared by reaction of 3'-bromo-3-methoxybiphenyl (**14ii**) (200 mg, 0.76 mmol), 5-methyl-2-thienyl boronic acid (129 mg, 0.91 mmol), caesium carbonate (991 mg, 3.04 mmol) and tetrakis(triphenylphosphine) palladium (26 mg, 23 μmol) according to method A. The product was purified by CC (hexane/ethyl acetate 99:1); yield: 94 % (200 mg); ^1H NMR (CD_3COCD_3): 7.84 (t, *J* = 1.9 Hz, 1H), 7.58 (ddd, *J* = 1.3 Hz and *J* = 1.9 Hz and *J* = 7.6 Hz, 1H), 7.55-7.53 (m, 1H), 7.46 (t, *J* = 7.6 Hz, 1H), 7.39 (t, *J* = 7.9 Hz, 1H), 7.34 (d, *J* = 3.5 Hz, 1H), 7.25 (ddd, *J* = 0.9 Hz and *J* = 1.6 Hz and *J* = 7.6 Hz, 1H), 7.23 (t, *J* = 2.1 Hz, 1H), 6.96 (ddd, *J* = 0.9 Hz and *J* = 2.6 Hz and *J* = 8.2 Hz, 1H), 6.82-6.80 (m, 1H), 3.88 (s, 3H), 2.50 (s, 3H); ^{13}C NMR (CD_3COCD_3): 162.30, 143.70, 143.40, 141.50, 131.80, 131.70, 131.40, 128.50, 127.70, 126.20, 125.70, 125.50, 121.20, 115.00, 114.80, 114.50, 56.60, 16.30.

3'-(5-Methyl-2-thienyl)biphenyl-3-ol (**14**). The title compound was prepared by reaction of 2-(3'-methoxybiphenyl-3-yl)-5-methylthiophene (**14i**) (200 mg, 0.71 mmol) and boron tribromide (2.13 mmol) according to method D. The product was purified by CC (hexane/ethyl acetate 99:1); yield: 95 % (180 mg); MS (ESI): 267 $(M+H)^+$; ^{1}H NMR (CD_3COCD_3): 8.42 (s, 1H), 7.80 (t, *J* = 1.6 Hz, 1H), 7.57-7.56 (m, 1H), 7.51-7.49 (m, 1H), 7.45 (t, *J* = 7.6 Hz, 1H), 7.33 (d, *J* = 3.5 Hz, 1H), 7.30 (t, *J* = 8.2 Hz, 1H), 7.16-7.15 (m, 2H), 6.86 (ddd, *J* = 0.9 Hz and *J* = 2.6 Hz and *J* = 8.2 Hz, 1H), 6.81-6.80 (m, 1H), 2.50 (s, 3H); ^{13}C NMR (CD_3COCD_3): 159.80, 144.10, 143.80, 143.50, 141.40, 137.10, 131.90, 131.40, 128.50, 127.60, 126.10, 125.50, 125.40, 120.10, 116.50, 115.80, 15.50; IR: 3486, 3375, 1575, 1184, 777, 692 cm^{-1}.

2-(3'-Methoxybiphenyl-3-yl)-1-benzothiophene (**15i**). The title compound was prepared by reaction of 3'-bromo-3-methoxybiphenyl (**14ii**) (200 mg, 0.76 mmol), benzothiophene-2-boronic acid (162 mg, 0.91 mmol), caesium carbonate (991 mg, 3.04 mmol) and tetrakis(triphenylphosphine) palladium (26 mg, 23 µmol) according to method A. The product was purified by CC (hexane/ethyl acetate 9:1); yield: 47 % (113 mg); ^{1}H NMR (CD_3COCD_3): 8.05 (t, *J* = 1.8 Hz, 1H), 7.95-7.94 (m, 1H), 7.89 (s, 1H), 7.86 (dd, *J* = 1.9 Hz and *J* = 7.2 Hz, 1H), 7.77 (ddd, *J* = 0.9 Hz and *J* = 1.9 Hz and *J* = 7.5 Hz, 1H), 7.66 (ddd, *J* = 1.3 Hz and *J* = 1.9 Hz and *J* = 7.9 Hz, 1H), 7.56 (t, *J* = 7.8 Hz, 1H), 7.43-7.35 (m, 3H), 7.31 (ddd, *J* = 0.9 Hz and *J* = 1.6 Hz and *J* = 7.5 Hz, 1H), 7.29 (t, *J* = 2.1 Hz, 1H), 6.98 (ddd, *J* = 0.9 Hz and *J* = 2.5 Hz and *J* = 8.2 Hz, 1H), 3.89 (s, 3H); ^{13}C NMR (CD_3COCD_3): 162.30, 143.80, 142.90, 141.20, 136.70, 131.90, 131.60, 130.70, 129.00, 127.30, 126.70, 126.60, 126.50, 125.70, 124.20, 122.20, 121.30, 115.20, 114.60, 56.70.

3'-(1-Benzothien-2-yl)biphenyl-3-ol (**15**). The title compound was prepared by reaction of 2-(3'-methoxybiphenyl-3-yl)-1-benzothiophene (**15i**) (113 mg, 0.36 mmol) and boron tribromide (1.08 mmol) according to method D. The product was purified by preparative TLC (hexane/ethyl acetate 8:2); yield: 77 % (83 mg); MS (ESI): 303 $(M+H)^+$; ^{1}H NMR (CD_3COCD_3): 8.45 (s, 1H), 8.01 (t, *J* = 1.8 Hz, 1H), 7.95-7.94 (m, 1H), 7.89 (s, 1H), 7.87-7.85 (m, 1H), 7.77 (ddd, *J* = 1.3 Hz and *J* = 1.9 Hz and *J* = 7.9 Hz, 1H), 7.62 (ddd, *J* = 0.9 Hz and *J* = 1.6 Hz and *J* = 7.6 Hz, 1H), 7.55 (t, *J* = 7.8 Hz, 1H), 7.41-7.31 (m, 3H), 7.22-7.20 (m, 2H), 6.90-6.88 (m, 1H); ^{13}C NMR (CD_3COCD_3): 171.90, 159.90, 143.90, 142.90, 141.20, 136.60, 131.90, 131.60, 128.90, 127.20, 126.70, 126.60, 126.50, 125.70, 124.20, 122.20, 120.20, 116.60, 115.80; IR: 3464, 3387, 1574, 1179, 779, 692 cm^{-1}.

3-[2-(2-Thienyl)pyridin-4-yl]phenol (**16**). Compound **16** was obtained as a side product of the reaction of compound **17**; yield: 22 % (17 mg); MS (ESI): 254 $(M+H)^+$; ^{1}H NMR (CD_3OD): 8.42 (dd, *J* = 0.6 Hz and *J* = 5.4 Hz, 1H), 7.91 (dd, *J* = 0.6 Hz and *J* = 1.6 Hz, 1H), 7.71 (dd, *J* =

0.9 Hz and *J* = 3.8 Hz, 1H), 7.46 (dd, *J* = 1.3 Hz and *J* = 5.1 Hz, 1H), 7.40 (dd, *J* = 1.6 Hz and *J* = 5.4 Hz, 1H), 7.27 (t, *J* = 7.9 Hz, 1H), 7.17 (ddd, *J* = 0.9 Hz and *J* = 1.9 Hz and *J* = 7.9 Hz, 1H), 7.11 (t, *J* = 2.1 Hz, 1H), 7.09 (dd, *J* = 3.5 Hz and *J* = 5.1 Hz, 1H), 6.84 (ddd, *J* = 0.9 Hz and *J* = 2.5 Hz and *J* = 8.2 Hz, 1H); ^{13}C NMR (CD_3OD): 159.40, 154.40, 151.40, 150.70, 145.30, 140.50, 131.40, 129.30, 128.90, 126.70, 121.30, 119.30, 118.20, 117.50, 114.80; IR: 3075, 1599, 1202, 781, 722, 692 cm^{-1}.

4-Bromo-2-(5-chloro-2-thienyl)pyridine (**17i**). The title compound was prepared by reaction of 2,4-dibromopyridine (300 mg, 1.27 mmol), 5-chloro-2-thienyl boronic acid (206 mg, 1.27 mmol), potassium carbonate (350 mg, 2.54 mmol) and tetrakis(triphenylphosphine) palladium (44 mg, 38 µmol) according to method B using 1 equivalent boronic acid. The product was purified by CC (hexane/ethyl acetate 99:1); yield: 24 % (83 mg); ^{1}H NMR (CD_3COCD_3): 8.36 (dd, *J* = 0.6 Hz and *J* = 5.4 Hz, 1H), 8.07 (dd, *J* = 0.6 Hz and *J* = 1.9 Hz, 1H), 7.70 (d, *J* = 4.1 Hz, 1H), 7.48 (dd, *J* = 1.9 Hz and *J* = 5.4 Hz, 1H), 7.08 (d, *J* = 4.1 Hz, 1H); ^{13}C NMR (CD_3COCD_3): 154.80, 152.40, 144.40, 134.90, 134.40, 129.80, 127.40, 127.30, 122.90.

3-[2-(5-Chloro-2-thienyl)pyridin-4-yl]phenol (**17**). The title compound was prepared by reaction of 4-bromo-2-(5-chloro-2-thienyl)pyridine (**17i**) (83 mg, 0.30 mmol), 3-hydroxybenzene boronic acid (50 mg, 0.36 mmol), caesium carbonate (391 mg, 1.20 mmol) and tetrakis(triphenylphosphine) palladium (11 mg, 9 µmol) according to method A. The product was purified by preparative TLC (hexane/ethyl acetate 6:4) followed by preparative HPLC; yield: 49 % (42 mg); MS (ESI): 288 $(M+H)^+$; ^{1}H NMR (CD_3OD): 8.43 (dd, *J* = 0.9 Hz and *J* = 5.4 Hz, 1H), 7.90 (dd, *J* = 0.9 Hz and *J* = 1.9 Hz, 1H), 7.56 (d, *J* = 4.1 Hz, 1H), 7.43 (dd, *J* = 1.9 Hz and *J* = 5.4 Hz, 1H), 7.28 (t, *J* = 7.9 Hz, 1H), 7.18 (ddd, *J* = 0.9 Hz and *J* = 1.9 Hz and *J* = 7.9 Hz, 1H), 7.11 (t, *J* = 1.9 Hz, 1H), 6.97 (d, *J* = 3.8 Hz, 1H), 6.85 (ddd, *J* = 0.9 Hz and *J* = 2.5 Hz and *J* = 8.2 Hz, 1H); ^{13}C NMR (CD_3OD): 159.40, 151.40, 150.80, 144.60, 140.40, 131.40, 128.80, 125.80, 121.60, 119.30, 118.30, 117.80, 117.50, 117.30, 114.80; IR: 3077, 1584, 1471, 799, 622 cm^{-1}.

2-(5-Chloro-2-thienyl)-6-(3-methoxyphenyl)pyridine (**18i**). The title compound was prepared by reaction of 2-bromo-6-(3-methoxyphenyl)pyridine (**18ii**) (250 mg, 0.95 mmol), 5-chloro-2-thienyl boronic acid (185 mg, 1.14 mmol), potassium carbonate (262 mg, 1.89 mmol) and tetrakis(triphenylphosphine) palladium (33 mg, 28 µmol) according to method B. The product was purified by CC (hexane/ethyl acetate 9:1); yield: 33 % (95 mg); ^{1}H NMR (CD_3COCD_3): 7.97 (dd, *J* = 0.6 Hz and *J* = 7.6 Hz, 1H), 7.80 (t, *J* = 7.8 Hz, 1H), 7.66-7.64 (m, 2H), 7.54 (dd, *J* = 0.6 Hz and *J* = 7.9 Hz, 1H), 7.41 (t, *J* = 8.2 Hz, 1H), 7.10 (d, *J* = 3.8 Hz, 1H), 7.05 (ddd, *J* = 0.9 Hz and *J* = 2.5 Hz and *J* = 8.2 Hz, 1H), 7.02 (d, *J* = 4.1Hz, 1H), 3.88 (s, 3H); ^{13}C NMR

(CD_3COCD_3): 162.20, 159.80, 143.50, 141.80, 136.80, 133.70, 130.30, 129.60, 129.40, 128.50, 125.80, 121.20, 120.90, 117.30, 114.00, 56.70.

3-[6-(5-Chloro-2-thienyl)pyridin-2-yl]phenol (**18**). The title compound was prepared by reaction of 2-(5-chloro-2-thienyl)-6-(3-methoxyphenyl)pyridine (**18i**) (95 mg, 0.32 mmol) and boron tribromide (0.96 mmol) according to method D. The product was purified by CC (hexane/ethyl acetate 8:2) followed by preparative HPLC; yield: 29 % (27 mg); MS (ESI): 288 $(M+H)^+$; ^{1}H NMR (CD_3OD): 7.83 (t, *J* = 7.9 Hz, 1H), 7.71 (dd, *J* = 0.6 Hz and *J* = 7.9 Hz, 1H), 7.68 (dd, *J* = 0.6 Hz and *J* = 7.9 Hz, 1H), 7.63-7.62 (m, 1H), 7.57-7.55 (m, 1H), 7.54 (d, *J* = 4.1 Hz, 1H), 7.33 (t, *J* = 7.9 Hz, 1H), 7.02 (d, *J* = 4.1 Hz, 1H), 6.90 (ddd, *J* = 0.9 Hz and *J* = 2.5 Hz and *J* = 8.2 Hz, 1H); ^{13}C NMR (CD_3OD): 159.00, 157.90, 152.70, 141.40, 139.00, 133.30, 133.20, 130.80, 129.80, 128.60, 125.10, 119.80, 119.10, 117.40, 114.70; IR: 3384, 1566, 1452, 1218, 775 cm^{-1}.

4-Bromo-2-(3-methoxyphenyl)pyridine (**19ii**). The title compound was prepared by reaction of 2,4-dibromopyridine (300 mg, 1.27 mmol), 3-methoxybenzene boronic acid (193 mg, 1.27 mmol), sodium hydrogencarbonate (213 mg, 2.54 mmol) and tetrakis(triphenylphosphine) palladium (44 mg, 38 µmol) according to method C. The product was purified by CC (hexane/ethyl acetate 96:4); yield: 44 % (147 mg); ^{1}H NMR (CD_3COCD_3): 8.54 (dd, *J* = 0.6 Hz and *J* = 5.4 Hz, 1H), 8.15-8.14 (m, 1H), 7.72-7.71 (m, 1H), 7.70-7.69 (m, 1H), 7.56 (dd, *J* = 1.9 Hz and *J* = 5.4 Hz, 1H), 7.41 (t, *J* = 7.8 Hz, 1H), 7.03 (ddd, *J* = 0.9 Hz and *J* = 2.5 Hz and *J* = 8.2 Hz, 1H), 3.88 (s, 3H); ^{13}C NMR (CD_3COCD_3): 162.20, 160.20, 152.50, 141.20, 134.90, 131.70, 127.40, 125.30, 121.10, 117.40, 114.00, 56.70.

4-(5-Chloro-2-thienyl)-2-(3-methoxyphenyl)pyridine (**19i**). The title compound was prepared by reaction of 4-bromo-2-(3-methoxyphenyl)pyridine (**19ii**) (147 mg, 0.56 mmol), 5-chloro-2-thienyl boronic acid (109 mg, 0.67 mmol), caesium carbonate (730 mg, 2.24 mmol) and tetrakis(triphenylphosphine) palladium (20 mg, 17 µmol) according to method A. The product was purified by CC (hexane/ethyl acetate 9:1); yield: 49 % (83 mg); ^{1}H NMR (CD_3COCD_3): 8.65 (dd, *J* = 0.6 Hz and *J* = 5.1 Hz, 1H), 7.80 (dd, *J* = 0.6 Hz and *J* = 1.6 Hz, 1H), 7.77-7.76 (m, 1H), 7.75-7.73 (m, 1H), 7.72 (d, *J* = 3.8 Hz, 1H), 7.49 (dd, *J* = 1.6 Hz and *J* = 5.1 Hz, 1H), 7.41 (t, *J* = 7.9 Hz, 1H), 7.16 (d, *J* = 4.1Hz, 1H), 7.02 (ddd, *J* = 0.9 Hz and *J* = 2.5 Hz and *J* = 8.2 Hz, 1H), 3.88 (s, 3H); ^{13}C NMR (CD_3COCD_3): 162.20, 152.30, 143.10, 142.30, 142.00, 132.80, 130.10, 127.80, 121.00, 120.40, 117.60, 116.80, 114.20, 56.60.

3-[4-(5-Chloro-2-thienyl)pyridin-2-yl]phenol (**19**). The title compound was prepared by reaction of 4-(5-chloro-2-thienyl)-2-(3-methoxyphenyl)pyridine (**19i**) (83 mg, 0.27 mmol) and

boron tribromide (0.81 mmol) according to method D. The product was purified by CC (hexane/ethyl acetate 8:2) followed by preparative HPLC; yield: 47 % (54 mg); MS (ESI): 288 $(M+H)^+$; ^{1}H NMR (CD_3SOCD_3): 8.63 (dd, *J* = 0.9 Hz and *J* = 5.4 Hz, 1H), 8.03 (dd, *J* = 0.9 Hz and *J* = 1.9 Hz, 1H), 7.85 (d, *J* = 4.1 Hz, 1H), 7.57-7.56 (m, 2H), 7.52 (dd, *J* = 1.9 Hz and *J* = 5.1 Hz, 1H), 7.30 (t, *J* = 8.1 Hz, 1H), 7.28 (d, *J* = 3.8 Hz, 1H), 6.86 (ddd, *J* = 0.9 Hz and *J* = 2.5 Hz and *J* = 8.2 Hz, 1H); ^{13}C NMR (CD_3SOCD_3): 157.70, 154.70, 140.60, 139.40, 129.60, 126.70, 118.00, 117.50, 116.30, 115.40, 113.50; IR: 3045, 1580, 1310, 780, 693 cm^{-1}.

3-(5-Chloro-2-thienyl)-5-(3-methoxyphenyl)pyridine (**20i**). The title compound was prepared by reaction of 3-bromo-5-(3-methoxyphenyl)pyridine (**20ii**) (60 mg, 0.23 mmol), 5-chloro-2-thienyl boronic acid (44 mg, 0.27 mmol), potassium carbonate (49 mg, 0.46 mmol) and tetrakis(triphenylphosphine) palladium (8 mg, 7 μmol) according to method B. The product was purified by CC (hexane/ethyl acetate 8:2); yield: 68 % (47 mg); ^{1}H NMR (CD_3COCD_3): 8.80 (t, *J* = 1.9 Hz, 2H), 8.18 (t, *J* = 2.2 Hz, 1H), 7.54 (d, *J* = 4.1 Hz, 1H), 7.43 (t, *J* = 8.2 Hz, 1H), 7.33-7.31 (m, 2H), 7.13 (d, *J* = 3.8 Hz, 1H), 7.03-7.01 (m, 1H), 3.89 (s, 3H); ^{13}C NMR (CD_3COCD_3): 169.00, 162.40, 149.30, 147.10, 140.50, 134.50, 133.00, 131.50, 131.30, 130.70, 129.90, 126.70, 121.30, 115.90, 114.60, 56.70.

3-[5-(5-Chloro-2-thienyl)pyridin-3-yl]phenol (**20**). The title compound was prepared by reaction of 3-(5-chloro-2-thienyl)-5-(3-methoxyphenyl)pyridine (**20i**) (120 mg, 0.40 mmol) and boron tribromide (1.20 mmol) according to method D. The product was purified by CC (hexane/ethyl acetate 8:2) followed by preparative HPLC; yield: 37 % (42 mg); MS (ESI): 288 $(M+H)^+$; ^{1}H NMR (CD_3OD): 8.79 (s, 1H), 8.75 (s, 1H), 8.27 (s, 1H), 7.51 (dd, *J* = 0.9 Hz and *J* = 3.8 Hz, 1H), 7.38 (t, *J* = 7.9 Hz, 1H), 7.20 (d, *J* = 7.9 Hz, 1H), 7.14 (s, 1H), 7.11 (dd, *J* = 0.9 Hz and *J* = 3.8 Hz, 1H), 6.94-6.92 (m, 1H); ^{13}C NMR (CD_3OD): 159.50, 132.30, 131.50, 129.20, 126.50, 119.50, 117.00, 115.50, 115.10; IR: 3188, 1589, 1329, 1014, 776, 693 cm^{-1}.

Biological methods

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

17β-HSD1 and 17β-HSD2 were obtained from human placenta according to previously described procedures.[23] Fresh human placenta was homogenized and cytosolic fraction and microsomes were separated by 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 17β-HSD1

Inhibitory activities were evaluated by an established method with minor modifications.[31] Briefly, the enzyme preparation was incubated with NADH [500 µM] in the presence of potential inhibitors at 37 °C in a phosphate buffer (50 mM) supplemented with 20 % of glycerol and EDTA (1 mM)**.** Inhibitor stock solutions were prepared in DMSO. The final concentration of DMSO was adjusted to 1 % in all samples. The enzymatic reaction was started by addition of a mixture of unlabelled- and [2, 4, 6, 7-^{3}H]-E1 (final concentration: 500 nM, 0.15 µCi). After 10 min, the incubation was stopped with $HgCl_2$ and the mixture was extracted with diethylether. After evaporation, the steroids were dissolved in acetonitrile. E1 and E2 were separated using acetonitrile/water (45:55) as mobile phase in a C18 reverse phase chromatography column (Nucleodur C18 Gravity, 3 µm, Macherey-Nagel, Düren) connected to a HPLC-system (Agilent 1100 Series, Agilent Technologies, Waldbronn). Detection and quantification of the steroids were performed using a radioflow detector (Berthold Technologies, Bad Wildbad). The conversion rate was calculated after analysis of the resulting chromatograms according to the following equation: $\%conversion = \frac{\%E2}{\%E2 + \%E1} \times 100$. Each value was calculated from at least three independent experiments.

Inhibition of 17β-HSD2

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

The conversion rate was calculated after analysis of the resulting chromatograms according to the following equation: $\%conversion = \frac{\%E1}{\%E1 + \%E2} \times 100$.

ER affinity

The binding affinity of selected compounds to the ERα and ERβ was determined according to Zimmermann et al.[49] Briefly, 0.25 pmol of ERα or ERβ, respectively, were incubated with [2, 4, 6, 7-^{3}H]-E2 (10 nM) and test compound for 1 h at room temperature. The potential inhibitors were dissolved in DMSO (5 % final concentration). Evaluation of non-specific-binding was performed with diethylstilbestrol (10 µM). After incubation, ligand-receptor complexes were selectively bound to hydroxyapatite (5 g/ 60 mL TE-buffer). The complex formed was separated, washed and resuspended in ethanol. For radiodetection, scintillator cocktail (Quickszint 212, Zinsser Analytic, Frankfurt) was added and samples were measured in a liquid scintillation counter (Rack Beta Primo 1209, Wallac, Turku). For determination of the relative binding affinity (RBA), inhibitor and E2 concentrations required to displace 50 % of the

receptor bound labelled E2 were determined. RBA values were calculated according to the following equation: $RBA[\%] = \frac{IC_{50}(E2)}{IC_{50}(compound)} \times 100$. The RBA value for E2 was arbitrarily set at 100 %.

Computational chemistry

Molecular Modeling. All calculations and graphical manipulations were performed on Intel(R) Core(TM)2 Duo CPU 3.00 GHz running Linux CentOS 5.3 while using the software packages AutoDock4.2[37] as implemented through the graphical user interface AutoDockTools1.5.4,[37] GOLDv4.0.1[38] and MOE2008.10 (Chemical Computing Group Inc., Montreal, Canada).

Atomic coordinates of 17β-HSD1 used during molecular modeling simulations were derived from the structure of the ternary complex between 17β-HSD1, $NADP^+$ and equilin (RCSB Protein Data Bank entry 1EQU).

To set the initial coordinates for the docking studies, chain A was isolated in the coordinate file and the cocrystallized equilin as well as all water molecules were removed. Missing protein atoms were added and correct atom types set. Ionisation states and hydrogen positions were assigned using the Protonate 3D utility of MOE. Furthermore the crystal structure was energy minimized with MOE applying MMFF94x force field and generalized Born model, keeping the coordinates of protein backbone atoms fixed.

Inhibitors were energy-minimized with MOE using the MMFF94x force field and the atomic partial charges for the inhibitors and the cofactor were calculated using the AM1-BCC approximation.

Two docking softwares, namely Autodock4.2 and GOLDv4.0.1, were used to evaluate the binding mode of the inhibitors.

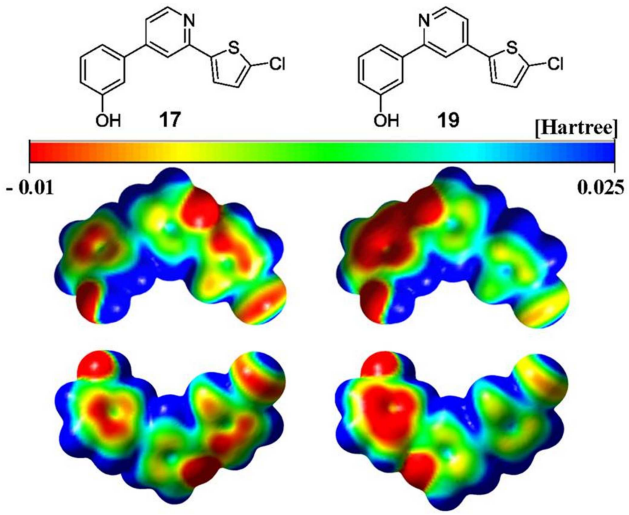
Autodock4.2. The docking area has been defined by a box, centered on the steroid binding site. Grid points of 86 x 80 x 80 with 0.250 Å spacing were calculated around the docking area for all the ligand atom types using AutoGrid4.2. For each inhibitor, 100 separate docking calculations were performed. Each docking calculation consisted of $25x10^5$ energy evaluations using the Lamarckian genetic algorithm local search (GALS) method. Each docking run was performed with a population size of 250. A mutation rate of 0.02 and a crossover rate of 0.8 were used to generate new docking trials for subsequent generations. The docking results from each of the 100 calculations were clustered on the basis of root-mean-square deviation (RMSD = 2.0Å) between the Cartesian coordinates of the ligand atoms and were ranked on the basis of the free binding energy.

GOLDv4.0.1. Active site origin was set at the center of the steroid binding site and a radius of 15 Å was chosen. The automatic active-site detection was switched on. Further, GOLDscore[38]

fitness function was used and genetic algorithm default parameters were set as suggested by the GOLD authors. For each of the selected compounds 50 solutions were generated and compounds were ranked according to GOLDscore.
Both programs performed in a similar way, supporting the herein suggested binding modes. The quality of the docked poses was evaluated based mainly on visual inspection of the putative binding modes of the ligand, and secondly on the scoring functions.

MEP. For selected compounds ab initio geometry optimizations were performed for the gas phase at the B3LYP/6-311++G (d,p) level of density functional theory (DFT) by means of the Gaussian 03 software, and the molecular electrostatics potential map (MEP) was plotted using GaussView, version 3.0, the 3D molecular graphics package of Gaussian.[50] These electrostatic potential surfaces were generated by mapping 6-311++G electrostatic potentials onto surfaces of molecular electron density (isovalue of 0.004 e/Å). The MEP maps are color-coded, where red stands for negative values (-1.0 x 10^{-2} Hartree) and blue for positive ones (2.5 x 10^{-2} Hartree).

MEP calculations in supporting material



MEP calculations of compounds 17 and 19
structure of compounds **17** and **19** with EP maps, dorsal and ventral view

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3.2 Bicyclic Substituted Hydroxyphenylmethanones as Novel Inhibitors of 17β-Hydroxysteroid Dehydrogenase Type 1 (17β-HSD1) for the Treatment of Estrogen-Dependent Diseases

Alexander Oster, Stefan Hinsberger, Ruth Werth, Sandrine Marchais-Oberwinkler, Martin Frotscher, Rolf W. Hartmann

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Abstract

Estradiol (E2), the most important estrogen in humans, is involved in the initiation and progression of estrogen-dependent diseases such as breast cancer and endometriosis. Its local production in the target cell is regulated by 17β-hydroxysteroid dehydrogenase type 1 (17β-HSD1), which catalyzes E2-formation by reduction of the weak estrogen estrone (E1). Because the enzyme is expressed in the diseased tissues, inhibition of 17β-HSD1 is considered as a promising therapy for the treatment of estrogen-dependent diseases. For the development of novel inhibitors, a structure- and ligand-based design strategy was applied resulting in bicyclic substituted hydroxyphenylmethanones. *In vitro* testing revealed high inhibitory potencies toward human placental 17β-HSD1. Compounds were further evaluated with regard to selectivity (17β-HSD2, estrogen receptors ERα and ERβ), intracellular activity (T47D cells) and metabolic stability. The most promising compounds, **14** and **15**, showed IC_{50}-values in the low nanomolar range in the cell-free and cellular assays (8 to 27 nM), more than 30-fold selectivity toward 17β-HSD2 and no affinity toward the ERs. The data obtained make these inhibitors interesting candidates for further preclinical evaluation.

Introduction

Breast cancer and endometriosis are estrogen-dependent diseases, which show a strong global prevalence. Breast cancer is one of the two leading causes of cancer death in women. Although

some therapeutic approaches are already available, the five-year survival rate for breast cancer patients only remains around 80 % (www.cancer.org). The Endometriosis Foundation of America has pointed out that endometriosis is one of the top three causes of female infertility (www.endofound.org). However, until recently, there has been no oral medication available to cure this disease.

The local production of estradiol (E2) plays a pivotal role for the initiation and development of both diseases.[1, 2] Thus, the reduction of tissue levels of active estrogens by inhibiting their synthetic pathway moves steadily to the forefront. Two therapeutic approaches have mainly been investigated within the past years. Aromatase inhibitors[3-8] (to reduce the transformation of androgens into estrogens) and sulfatase inhibitors[9] (to reduce the conversion of estrone sulfate (E1S) into estrone (E1)) intervene in the penultimate step of E2 biosynthesis. Both therapeutic approaches show disadvantages by their rather radical reduction of systemic estrogen concentration. A softer therapy seems to be the inhibition of 17β-hydroxysteroid dehydrogenase type 1 (17β-HSD1). This enzyme regulates the ratio of the weak active estrogen E1 and its potent metabolite E2 by catalyzing the reduction of E1 to E2 using NAD(P)H as a cofactor (Chart 1).

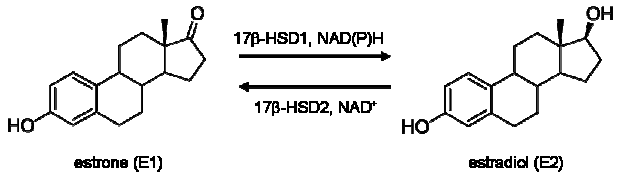


***Chart 1*: Interconversion of estrone (E1) and estradiol (E2)**

Interestingly, mRNA of 17β-HSD1, which is also used as a prognostic marker, is often overexpressed in breast cancer tissues[10, 11] and endometriotic lesions.[12] Considering the fact that therapeutic approaches regulating intracellular hormone concentrations have already been used successfully in androgen dependent diseases such as benign prostatic hyperplasia and alopecia,[13-16] 17β-HSD1 appeared to be a promising target for the local reduction of E2 and accordingly for the treatment of breast cancer and endometriosis. In contrast to the applied therapies, a basal estrogenic activity will be maintained because the E1 level should not be affected. Thus, side effects caused by a total absence of estrogenic activity could be avoided. The tissue specific influence of 17β-HSD1 inhibitors seems especially suitable for the therapy of postmenopausal women, in which the local production (e.g., in breast or endometrium) represents the major source of E2.

As it has recently been suggested, 17β-HSD1 is also involved in the metabolism of retinoic acid,[17, 18] a natural compound which is known to have antitumor activity.[19] Thus, inhibition of 17β-HSD1 should have an additional beneficial effect.

Efficacy of 17β-HSD1 inhibitors has been shown by several groups in different animal models. Husen et al.[20, 21] and Day et al.[22] used xenograft models with MCF7-breast cancer cells stably expressing recombinant human 17β-HSD1 or the breast cancer cell line T47D, respectively, in order to demonstrate *in vivo* efficacy of their inhibitors by decreasing tumor growth. In transgenic mice, it was proven that 17β-HSD1 inhibitors are able to reduce the conversion of E1 into E2.[23] With regards to endometriosis, two *in vivo* models have been established.[24, 25] Using nude mice implanted with endometrial tissue from human donors, the expression of steroid hormone receptors and steroid converting enzymes, as well as the proliferation of the ectopic tissue, can be analyzed.[24] In a second model, endometriosis is induced in marmoset monkeys, either by endometrial reflux or invasively by laparotomy.[25] Despite encouraging *in vivo* data, no 17β-HSD1 inhibitor has entered clinical evaluation yet. Thus, the applied inhibitors do not seem to be appropriate candidates for clinical application.

The therapeutic concept requires selectivity of potent inhibitors toward 17β-hydroxysteroid dehydrogenase type 2 (17β-HSD2) because it catalyzes the reverse reaction, the oxidation of E2 into E1. Furthermore, to reduce the risk of intrinsic estrogen effects, affinity toward both estrogen receptors α and β (ERα and ERβ) should be avoided, even if the pathophysiological role of ERβ is not completely clarified.

The selective inhibition of 17β-HSD1 was the goal of several groups investigating inhibitors over the past years.[18, 26-30] Their drug design efforts were facilitated by the existence of several X-ray structures of human 17β-HSD1 and will be further alleviated by the recently published first insights in 17β-HSD1 enzyme kinetics and ligand binding by dynamic motion investigations.[31] Although running the higher risk of unwanted side effects, most of these groups focused on the development of compounds bearing a steroidal scaffold. Regarding non-steroidal inhibitors, aside from phytoestrogens[27, 32] and thiophenepyrimidinones,[33, 34] there are two additional compound classes showing inhibitory activity in the low nanomolar range, namely the (hydroxyphenyl)naphthols[35-39] and the bis(hydroxyphenyl)heterocycles.[40-45] Recently, we also reported on heterocyclic substituted biphenylols,[46] which demonstrate notable inhibitory activity. Molecular modeling studies indicated that linear and angulate molecules out of this class unfold their activity via interaction with the catalytic center and a rather lipophilic subpocket located below the catalytic site, respectively.[46]

In the following, we will report on the design, synthesis, and biological evaluation of a novel class of 17β-HSD1 inhibitors, namely bicyclic substituted hydroxyphenylmethanones.

Design

Molecular docking studies of the most potent heterocyclic substituted biphenylol inhibitors **A** and **B** (Chart 2) using the crystal structure 1EQU (PDB-ID) indicated that their heterocyclic part

interacts with different areas of the active site.[46] Designed using E1 as a template, compound **A** shows the same interaction pattern as the natural substrate. In detail: the *meta*-OH-phenyl ring imitates the steroidal A-ring and the methoxypyridine interacts as hydrogen-bond acceptor with Ser142 and Tyr155, which together with Asn114 and Lys159 constitute the catalytic tetrade. While the OH-phenyl ring of **B** also mimics the steroidal A-ring, its chlorothiophene part points in the direction of a mainly apolar subpocket consisting of Leu95, Leu96, Asn152, Tyr155 and Phe192.

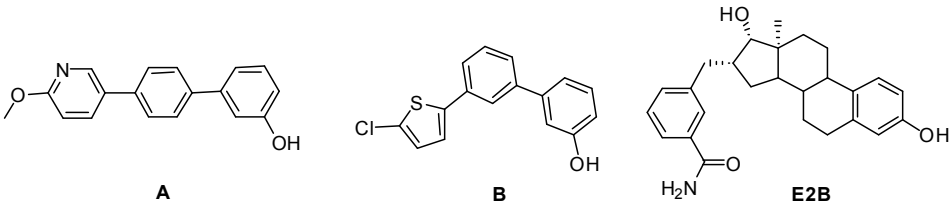
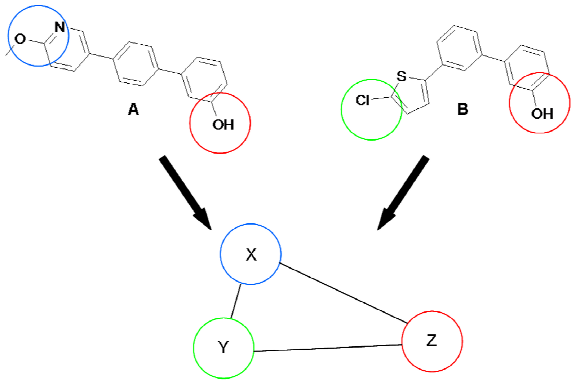


***Chart 2*: Most potent 17β-HSD1 Inhibitors of heterocyclic substituted biphenylol compound class (compound A and B) and E2B**

Our strategy focuses on the design of inhibitors, which fit into a three-point-pharmacophore model derived from the different interactions found for **A** and **B** (Chart 3). A closer analysis of the amino acids in these regions by computational docking studies provides ideas for substituents as appropriate interacting partners as well as for the structural architecture of the compounds.



X = pure hydrogen bond acceptor (ketone): interactions in catalytic center with Tyr155 + Ser142
Y = H-bond donor + lipohilic moiety (phenol): interactions in lipophilic subpocket with Asn152
Z = H-bond donor and/or H-bond acceptor (OH): interactions in C-terminal region with His221 + Glu282

***Chart 3*: Design concept and pharmacophore model**

To mimic the interactions established by the steroidal A-ring of the substrate with the amino acids His221 and Glu282, a hydroxy-substituted phenyl moiety will be one important component of our inhibitors. The second part should interact with the catalytic tetrade. Inhibitors with two H-bond acceptor groups located in this region are described to inhibit 17β-HSD1.[46] One functional group with two hydrogen bond acceptor properties could eventually be

more appropriate to target the corresponding amino acids. A keto-group, as in the natural substrate E1, should be the functionality of choice. Moreover, it disrupts the linearity present in **A**, thus allowing for binding with a third interaction region, which is constituted by a mainly apolar subpocket located beneath the catalytic center. In this subpocket, Asn152 is the only hydrophilic amino acid. This amino acid was already exploited for active compound design: Recently, Mazumdar et al.[47] reported on the crystal structure of the steroidal inhibitor **E2B** (Chart 2), whose *meta*-amidobenzyl substituent was found to interact with Asn152. Consequently, a second OH-phenyl moiety was introduced as a further component of the inhibitor structure in order to fit in this apolar subpocket and interact with Asn152 (Chart 4).

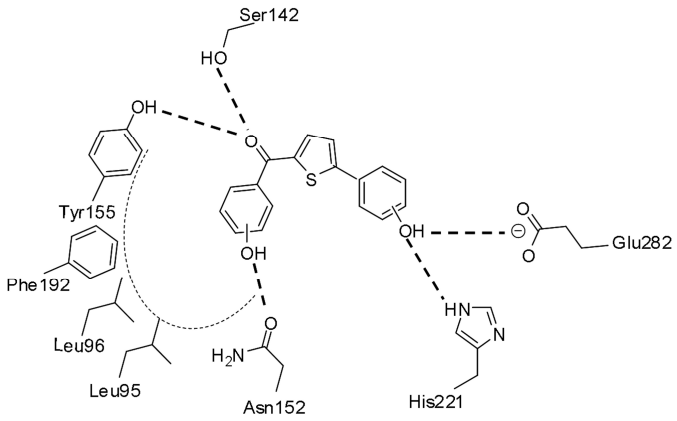


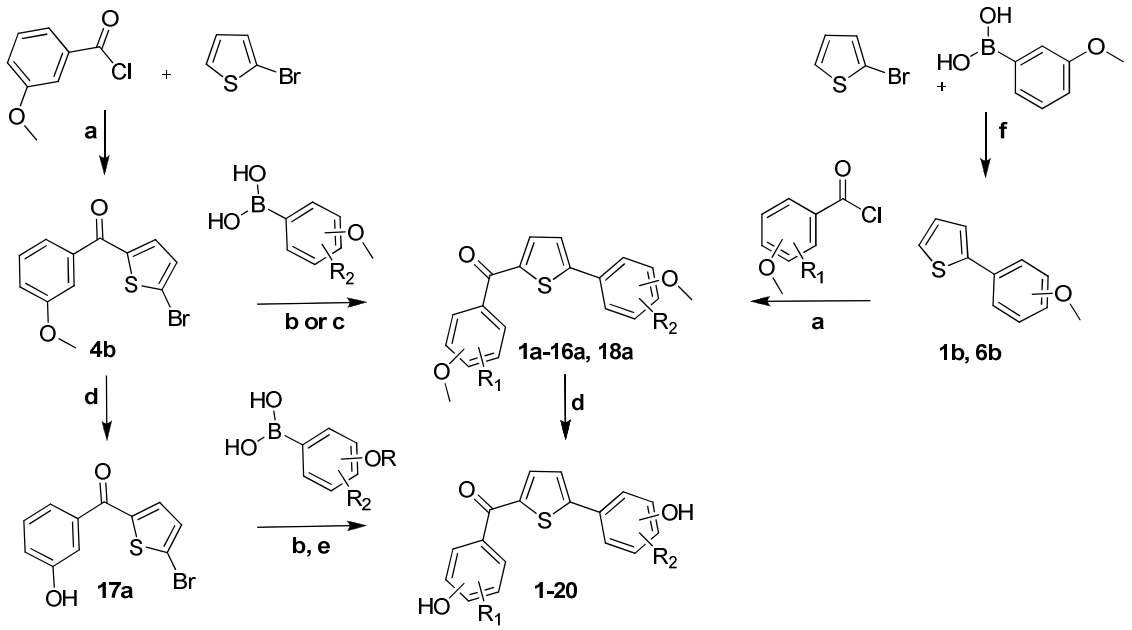
Chart 4: Schematic representation of the inhibitor template binding to the amino acids of the active site (possible hydrogen bond interactions are depicted in dashed lines)

The design concept resulted in compounds **1-5**, which were selected as the starting point for this study. Thiophene was chosen as the connecting group between the hydroxyphenyl and the keto-group in order to fit in the rather lipophilic substrate binding site (known as hydrophobic tunnel) and to fit the distance between the various interacting partners in the protein. After identifying **1** and **4** as most promising, small substituents were introduced into these compounds (**6-20**). Finally, the substitution pattern of the thiophene was changed (**21**) and it was replaced by thiazole (**22-24**).

Chemistry

The synthesis of the thiophene derivatives **1-20** is depicted in Scheme 1. The first step in the preparation of compounds **1-3** and **6-10** was the coupling of 2-bromothiophene with 3-methoxy- and 4-methoxybenzeneboronic acid, respectively, thus leading to intermediates **1b** and **6b**. A Friedel-Crafts acylation (method A) was then performed with the appropriate benzoyl chlorides, resulting in compounds **1a-3a** and **6a-10a**. Subsequently, treatment with boron tribromide led to the corresponding dihydroxylated final compounds **1-3** and **6-10**.

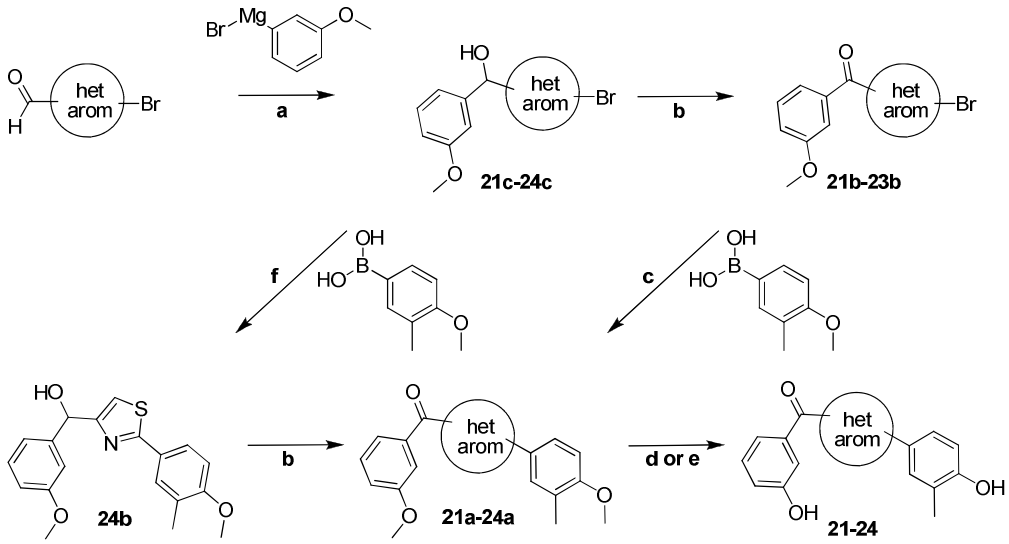
The brominated key intermediate **4b** was synthesized via Friedel-Crafts-acylation of 2-bromothiophene with 3-methoxybenzoyl chloride. Using **4b**, Suzuki cross coupling reactions[48] with the appropriate commercially available boronic acids were carried out via two different methods (B for compounds **14a-16a** and C[41] for compounds **4a**, **5a**, **11a-13a** and **18a**, scheme 1). The methoxy functions were cleaved with BBr_3[49] yielding compounds **4**, **5**, **11-16** and **18**.
Ether cleavage of the key intermediate **4b** gave **17a** in quantitative yield. Cross coupling of **17a** with the appropriate hydroxylated benzene boronic acids resulted in the dihydroxylated compounds **17** and **19**.
3-Ethyl-4-methoxybenzeneboronic acid was synthesized using the method of Allan et al.,[50] and was used for Suzuki reaction in order to yield the monomethoxy derivative **20a**. The latter was heated with pyridinium hydrochloride[51] to yield compound **20**.



***Scheme 1*: Synthesis of compounds 1-20**[a]

[a] reagents and conditions: (a) Method A: $AlCl_3$, anhydrous CH_2Cl_2, 0 °C, 0.5 h and then rt, 1 h, for compounds **1a-3a**, **4b**, **6a-10a**; (b) Method B: Cs_2CO_3, $Pd(PPh_3)_4$, DME/water (1:1), reflux, 2 h, for compounds **14a-16a**, **17**, **19**, **20a**; (c) Method C: Cs_2CO_3, $Pd(PPh_3)_4$, DME/EtOH/water (1:1:1), microwave conditions (150 W, 15 bar, 150 °C, 15 min), for compounds **4a**, **5a**, **11a-13a**, **18a**; (d) Method D: BBr_3, CH_2Cl_2, -78 °C, 20 h, for compounds **1-16**, **17a**, **18**; (e) Method E: pyridinium hydrochloride, 220 °C, 18 h, for compound **20**; (f) Na_2CO_3, $Pd(PPh_3)_4$, THF/water (1:1), reflux, 18 h, for compound **1b**

The preparation of compounds **21-24** (Scheme 2) started with a Grignard reaction using 3-methoxyphenylmagnesium bromide and the appropriate brominated aromatic carbaldehydes (method F). The OH-groups of compounds **21c-23c** were subsequently oxidized to the keto-functions with 2-iodoxybenzoic acid (IBX) (method G). Suzuki cross coupling reactions using the 4-methoxy-3-methylbenzeneboronic acid and the brominated methanones **21b-23b** led to compounds **21a-23a**. Demethylation with BBr_3 (method D) yielded the final compounds **21** and **23**, whereas pyridinium hydrochloride (method E) was used for the preparation of **22**.



***Scheme 2*: Synthesis of compounds 21-24**[a]

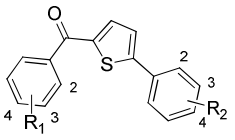
[a] reagents and conditions: (a) Method F: anhydrous THF, 80 °C, 3 h, for compounds **21c**-**24c**; (b) Method G: 2-iodoxybenzoic acid, anhydrous THF, 0 °C, 10 min and 60 °C, 18 h, for compounds **21b**-**23b**, **24a**; (c) Method B: Cs_2CO_3, $Pd(PPh_3)_4$, DME/water (1:1), reflux, 2 h, for compounds **21a**-**23a**; (d) Method D: BBr_3, CH_2Cl_2, -78 °C, 20 h, for compounds **21**, **23**; (e) Method E: pyridinium hydrochloride, 220 °C, 18 h, for compounds **22**, **24**; (f) Cs_2CO_3, $Pd(PPh_3)_4$, DME/water (1:1), reflux, 4 h, for compound **24b**

For the preparation of **24**, Suzuki coupling was performed using the hydroxylated intermediate **24c** because its oxidation failed. Subsequently, **24b** was oxidized by means of IBX in anhydrous THF. Reaction of **24a** with pyridinium hydrochloride led to **24**.

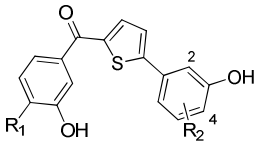
Biological results

Activity: Inhibition of human 17β-HSD1

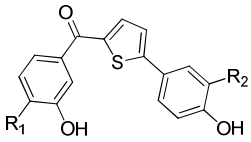
Placental enzyme was isolated following a described procedure[38] and incubated with tritiated E1, cofactor, and inhibitor. After HPLC separation of substrate and product, the amount of labeled E2 formed was quantified. The inhibition values of compounds **1**-**24** are presented in Tables 1 and 2.



1-5



8-13



6, **7**, **14-20**

cmpd	R_1	R_2	IC_{50} [nM][a]		SF[d]
			17β-HSD1[b]	17β-HSD2[c]	
1	3-OH	3-OH	22	109	5
2	4-OH	3-OH	368	376	1
3	2-OH	3-OH	945	567	0.6
4	3-OH	4-OH	33	478	14
5	3-OH	2-OH	95	18	0.2
1			22	109	5
8	CH_3		64 %[e]	31 %[e]	
9	F		18	49	3
10	NO_2		594	240	0.4
11		4-CH_3	207	465	2
12		4-F	30	57	2
13		2-F	6	19	3
4			33	478	14
6	CH_3		52 %[e]	13 %[e]	
7	F		21	69	3
14		CH_3	8	382	48
15		F	19	588	31
16		CF_3	16	95	6
17		Cl	5	246	49
18		OH	86	590	7
19		OCH_3	108	793	7
20		CH_2CH_3	20	786	39

***Table 1*: Inhibition of human 17β-HSD1 and 17β-HSD2 by compounds 1-20**

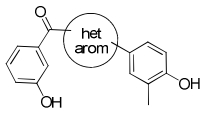
[a] Mean value of three determinations, standard deviation less than 23 %
[b] Human placenta, cytosolic fraction, substrate E1, 500 nM, cofactor NADH, 500 µM
[c] Human placenta, microsomal fraction, substrate E2, 500 nM, cofactor NAD^+, 1500 µM
[d] Selectivity factor: IC_{50} (17β-HSD2) / IC_{50} (17β-HSD1) [e] Inhibition at 1 µM (inhibitor concentration)

Compounds **1-5** with their keto and two OH-functions were designed to interact with three distinct areas of the active site. Among these inhibitors, the *meta-meta* substituted **1** showed the best IC_{50}-value (22 nM), demonstrating that in this class of compounds, the *meta*-OH substituted phenyl moiety is also very relevant for 17β-HSD1 inhibition as observed with the bis(hydroxyphenyl)heterocycles.[41] Displacement of the hydroxy-group on the benzoyl ring led to a decrease in activity (**2** and **3**). The position of the OH-group at the phenyl ring has a smaller impact on inhibitory activity: *para*-OH **4** (IC_{50} = 33 nM) was equipotent to *meta*-OH **1**, the potency of *ortho*-OH **5** was only slightly reduced (IC_{50} = 95 nM). The best substitution patterns identified in **1** (*meta-meta*) and **4** (*meta-para*) were sustained in the design of the further compounds. To examine the influence of additional small substituents in *para*-position of the benzoyl group, compounds **6-10** were synthesized. Introduction of a methyl substituent

decreased the inhibitory activity in both series strongly (**6** and **8**). An exchange of methyl by fluorine did not affect inhibition (**7** and **9**).

Introduction of methyl in *para*-position of the phenyl ring led to a 10-fold decrease in activity (**11**), whereas *meta*-CH_3, *para*-OH **14** turned out to be a very potent compound (IC_{50} = 8 nM). Fluorinated compounds with different substitution patterns showed a similarly high inhibition of 17β-HSD1 (IC_{50}: **12**, 30 nM; **13**, 6 nM; **15**, 19 nM). It is remarkable that the substitution with fluorine, in contrast to methyl, does not decrease the high inhibitory potency of the parent compounds **1** and **4**, regardless of their position at the benzoyl or phenyl ring. To provide more insight into the role of different substituents, **14** and **15** were used as starting points for further structural modifications. To combine the characteristics of methyl and fluorine, a CF_3-group (**16**), as well as chlorine (**17**) as its bioisosteric equivalent, was introduced. Both inhibitors confirmed the expected high inhibitory activity with IC_{50}-values of 14 nM and 5 nM, respectively. In contrast to this finding, a slight decrease in activity was caused by the replacement of the CH_3 for a hydroxy (**18**: IC_{50} = 86 nM) or a methoxy (**19**: IC_{50} = 108 nM). Interestingly, the ethyl derivative **20** once again revealed a high biological potency (IC_{50} = 20 nM), thus indicating that an oxygen in this position impinges on enzyme affinity.

Originally chosen as a suitable lipophilic component to fit the mainly apolar substrate binding site, we replaced the 2,5-disubstituted thiophene with 2,4-disubstituted thiophene and thiazole (**21**-**24**) in order to investigate the relevance of the middle ring on inhibitory activity. In the structurally related class of bis(hydroxyphenyl)heterocycles, a strong correlation between the nature of the heterocycle and the affinity toward 17β-HSD1 was already shown.[41] As **14** is one of the most active inhibitors in this series, its substitution pattern was maintained for this study. The biological results of compounds **21**-**24** are shown in Table 2.



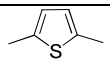
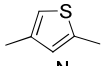
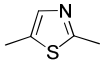
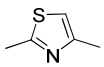
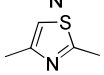
cmpd	structure	IC_{50} [nM][a]		SF[d]
		17β-HSD1[b]	17β-HSD2[c]	
14		8	382	48
21		199	719	4
22		35	485	14
23		32 %[e]	51 %[e]	
24		19 %[e]	57 %[e]	

***Table 2*: Inhibition of human 17β-HSD1 and 17β-HSD2 by compounds 14, 21-24**

[a] Mean value of three determinations, standard deviation less than 14 %
[b] Human placenta, cytosolic fraction, substrate E1, 500 nM, cofactor NADH, 500 µM
[c] Human placenta, microsomal fraction, substrate E2, 500 nM, cofactor NAD^+, 1500 µM
[d] Selectivity factor: IC_{50} (17β-HSD2) / IC_{50} (17β-HSD1) [e] Inhibition at 1µM (inhibitor concentration)

With regard to 2,4-disubstituted **21**, the decrease of activity (IC_{50} = 199 nM) underlines the importance of the sulfur position. Exchanging thiophene with thiazole led to compounds **22-24**. Compound **22**, which bears the sulfur of the thiazole in the same position as **14**, the thiophene-sulfur, still demonstrated a very good activity (IC_{50} = 35 nM). Interestingly, the potency of **23** and **24** strongly decreased, thereby confirming that the position of the sulfur plays a pivotal role for 17β-HSD1 inhibition.

Selectivity: Inhibition of human 17β-HSD2 and affinities to ERα and ERβ

Inhibition of 17β-HSD type 2 must be avoided, as it acts as a biological counterpart by catalyzing the reverse reaction: oxidation of E2 to E1. Compared with the type 1 enzyme, inhibition of 17β-HSD2 was determined in a similar assay using placental microsomes. These were incubated with tritiated E2 in the presence of NAD^+ and the inhibitor. The separation and quantification of the labeled product was performed by HPLC using radiodetection. IC_{50}-values and the percentage of inhibition, respectively, are shown in Tables 1 and 2. *In vitro* selectivity toward 17β-HSD2 is also expressed as selectivity factor (SF) describing the ratio of the concentration required to inhibit the activity of the isoenzymes by 50 % (IC_{50} 17β-HSD2 / IC_{50} 17β-HSD1).

Regarding compounds **1-5**, it became apparent that just the modification of the OH-substitution pattern influences the selectivity toward the type 2 enzyme. While compound **4** showed the best SF (14) out of these five, compounds **3** and **5**, which bear one of their OH-functions in the *ortho*-position, turned out to be potent inhibitors of 17β-HSD2 (SF = 0.6 and SF = 0.2, respectively). Introduction of further substituents on the benzoyl ring did not strongly influence the SF, with the exception of compound **10**, whose nitro group is also responsible for the reverse selectivity in favor of 17β-HSD2 (SF = 0.4). Concerning the different phenyl ring modified compounds, insertion of CH_3 (**14**, SF = 48) and F (**15**, SF = 31) in the *meta*-position (*ortho* to OH) increased selectivity compared to the unsubstituted compound **4 (**SF = 14). While other positions had less of a high impact (**11-13**), this *meta*-position seems to be a key position for 17β-HSD1 activity and selectivity toward 17β-HSD2. Chlorine (**17**) and ethyl (**20**) showed comparable high selectivities (SFs = 49 for **17** and 39 for **20**). The fact that chlorine and trifluoromethyl substituents have comparable electronic influences on a phenyl ring implies that other parameters are responsible for the difference in selectivity between 17β-HSD1 and 2.

Regarding the influence of the different middle rings on selectivity toward 17β-HSD2, it is striking that when compared with **14**, the isomeric thiophene **21** showed a strong reduction of selectivity. Only thiazole **22** showed a comparatively high SF of 14. Surprisingly, thiazoles **23** and **24** turned out to be more suitable inhibitors for 17β-HSD2, thereby demonstrating that the nature of the middle ring has a major impact on activity and selectivity.

To keep the risk of unwanted side effects as low as possible, intrinsic estrogenic effects should be avoided. Therefore, it is a prerequisite for 17β-HSD1 inhibitors to show no or low affinity for the ERs. Relative binding affinities (RBA) of compounds, which showed a selectivity factor higher than 10, were determined using recombinant human protein in a competitive binding assay applying tritium labeled E2 and hydroxyapatite. Because RBA of E2 is equated with 100 %, compounds showing a RBA-value below 0.1 % are considered to have neglectable receptor affinity. Within this study, none of the evaluated inhibitors crossed this value, neither for ERα nor ERβ.

Further biological evaluations

Besides the cell-free assay, T47D, a breast cancer cell line reflecting the conditions in many breast tumors by expressing subtypes 1 and 2 of 17β-HSD, was used to determine the intracellular potency of compounds having a SF higher than 10 (Table 3). While inhibitors **4**, **14**, and **15** confirmed their strong activity in T47D-cells with IC_{50}-values in the low nanomolar range (between 17 and 30 nM), compounds **17** and **22** showed inhibitory activities in the 100 nM range. Compared to the cell-free assay, only chloro- (**17**) and ethyl-derivative (**20**) demonstrated a remarkable reduction of inhibitory activity in T47D-cells.

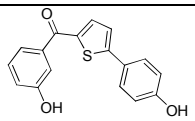
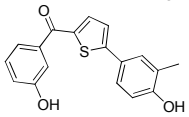
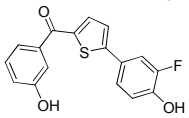
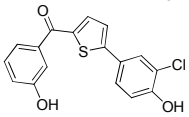
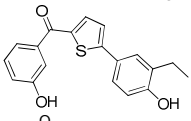
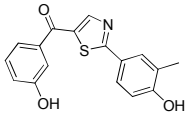
cmpd	structure	IC_{50} [nM][a]	
		cell-free assay[b]	cellular assay[c]
4		33	30
14		8	27
15		19	17
17		5	126
20		20	367
22		35	107

***Table 3*: Inhibition of 17β-HSD1 in cell-free and cellular assay by selected compounds**

[a] Mean values of three determinations, standard deviation less than 23 %
[b] Human placental, cytosolic fraction, substrate E1, 500 nM, cofactor NADH, 500 µM
[c] T47D cells, substrate E1, 50 nM

Additionally, metabolic stability of compounds **14**, **17**, and **22**, was evaluated using human liver microsomes. The corresponding data of the selected compounds and the reference compounds dextromethorphan and verapamil are presented in Table 4. All three inhibitors can be classified in the medium clearance category according to their half-life times around 100 min and thus are considered to have a reasonable metabolic stability. None of the small variations among these three inhibitors caused considerable differences in liability of the compounds to metabolic degradation.

cmpd	CLint [µL/min/mg protein]	$t_{1/2}$ [min]
dextromethorphan[a]	10.7	129
verapamil[a]	105	13.2
14[a]	13.8	100
17[b]	12.9	108
22[a]	17.0	81.4

***Table 4*: Metabolic stability of compounds 14, 17 and 22 and reference compounds using human liver microsomes**

[a] Test concentration = 3 µM
[b] Test concentration = 0.5 µM

Discussion

The results of molecular modeling studies in the class of heterocyclic substituted biphenylols[46] provided the basis for a successful design strategy using a new three-point-pharmacophore model. The determined biological results place emphasis on the validity of this structure- and ligand-based design approach, resulting in compound **1** as a new lead for further optimization. Interestingly, the position of a hydroxy-function at the benzoyl ring has more influence on inhibitory activity than the one at the phenyl ring. This suggests that either the expected hydrogen-bond interactions of the latter with His221 and Glu282 are less important for 17β-HSD1 inhibition or that the protein is flexible enough, at least in this part of the enzyme, to allow equal interaction with each derivative **1**, **4**, and **5**. In contrast to previous studies,[43] an exchange of this OH-phenyl moiety might also lead to highly active 17β-HSD1 inhibitors.

The introduction of further substituents, such as methyl and fluorine in different positions on the benzoyl and phenyl ring, resulted in interesting modulations of biological activities. Compounds **6** and **8**, bearing the methyl-substituent in the *para*-position of the benzoyl ring, showed a strong decrease of inhibitory activity, once again emphasizing the sharp structure-activity relationships within the rather apolar subpocket. There is plausibly a sterical hindrance caused by the CH_3-group, thus leading to a shifted position of the molecule that is unable to establish suitable hydrogen bond interactions. Taking into account that the geometrical radius of fluorine

is smaller than methyl, the fluorine substituted hydroxyphenyl-part of compounds **7** and **9** obviously still fits well into the subpocket, as can be seen by their high inhibitory activities.

Regarding the hydroxyphenyl, the introduction of methyl and fluorine in *ortho*- to the *para*-OH-substituent (**14** and **15**) improved inhibitory activity and even selectivity toward 17β-HSD2. It is striking that derivatives with more polar substituents (OH in compound **18** and OCH_3 in compound **19**) in this position could not exert such a high inhibitory activity, and especially their selectivity factor decreased. The fact that the Cl- and CH_2CH_3-compounds **17** and **20** have similar inhibitory properties comparable to those of **14** and **15**, led to the assumption that the amino acids surrounding this region of the 17β-HSD1 binding site are predominantly lipophilic. Only compound **16**, which bears a CF_3-group, demonstrated a low selectivity factor because of its strong inhibition of 17β-HSD2. The ethyl derivative **20**, which has a marginally lower SF than **14**, revealed a slightly reduced 17β-HSD2 inhibition, indicating that an enlargement of this lipophilic substituent might lead to lower inhibition of the type 2 enzyme.

Concerning selectivity toward ERα and ERβ, each inhibitor out of this new compound class showed neglectable affinity to the ERs, which confirms our strategy of developing non-steroidal inhibitors. Despite the fact that the keto-function and one hydroxy-group of the bicyclic substituted hydroxyphenylmethanones have to be considered as mimicks of the oxygen functions of E1, obviously, the absence of the sterane core and the additional phenolic ring are the main responsible factors that we succeeded in obtaining compounds without ER-affinity.

While the presence of the keto linker is the only structural difference between this new class of 17β-HSD1 inhibitors and the previously described bis(hydroxyphenyl)heterocycles,[40-43] several structure-activity relationships are in accordance. For example, the influence of the (hetero)aromatic middle ring is comparable. The exchange of the 2,5-disubstituted thiophene with 2,4-disubstituted thiophene **21** or different thiazoles **22**-**24** revealed that the position of the sulfur plays a critical role for enzyme inhibition. However, only compounds in which the sulfur is located between the two substituents show inhibition values in the low nanomolar range. Because it was shown that 2,4-thiophenes are also able to exhibit strong 17β-HSD1 inhibition,[41] the molecular electrostatic potential of the inhibitors can be discussed as a possible reason for the differences in inhibitory activity.[41] With regards to thiazoles, the corresponding compounds in the class of bis(hydroxyphenyl)heterocycles also revealed similar results. In both studies, there is a rather radical loss of inhibitory activity for all thiazole derivatives with nitrogen located between the two substituents, thus pointing out that this substitution pattern of thiazoles is detrimental for 17β-HSD1 inhibition. Interestingly, while compound **22** resembles the best inhibitor **14** in activity and selectivity, compounds **23** and **24** inhibited significantly stronger 17β-HSD2 than the type 1 enzyme. As there is neither an X-ray structure nor an applicable homology model of 17β-HSD2 available, these results cannot be interpreted on a sound basis.

The most important advantage of this compound class is its high cellular inhibitory activity. While most of the described intracellularly active 17β-HSD1 inhibitors were tested using very low substrate concentrations (2-30 nM),[33, 50] the new compound class revealed IC_{50}-values in the low nanomolar range in spite of using a much higher substrate concentration of 50 nM. Evaluated in our assay, the most potent inhibitors of the bis(hydroxyphenyl)heterocycles showed cellular inhibitory activities around 300 nM (IC_{50}).[43] Therefore, the keto-function seems to have a positive influence on cell permeation, protein binding, and/or intracellular metabolism. Only compounds **17** and **20** showed intracellularly a reduction of inhibitory activity compared to the cell-free assay. Increased lipophilicity might be responsible for this finding.

Concerning the metabolic stability study, the tested inhibitors **14**, **17**, and **22** revealed a medium intrinsic clearance confirming a reasonable metabolic profile. The fact that all three inhibitors showed comparable profiles indicates that neither the exchange thiophene-thiazole nor a variation of the additional substituent next to the hydroxy-function can give rise to some significant metabolic liability.

In this paper, we described the structure-based design approach, synthesis, and biological evaluations of bicyclic substituted hydroxyphenylmethanones as new highly potent and selective inhibitors of 17β-HSD1. The applied design concept is based on the newly built pharmacophore model, which resulted in compound **1** as the starting point of the study. The position of the hydroxy-functions, the influence of further small substituents and an alternative central ring were investigated. Structural optimization of this lead led to the development of **14**, **15**, **17**, and **22**, all of which show inhibitory activities in the very low nanomolar range in cell-free and cellular assays, remarkable selectivity towards 17β-HSD2, no affinity on both ERs, and very good metabolic stability (proven in case of **14**, **17** and **22**) tested on human liver microsomes. Hence, they represent promising 17β-HSD1 inhibitors, which fulfill all prerequisites for consideration as clinical candidates. Nevertheless, further optimizations appear possible, especially with regards to the phenyl ring and will therefore be investigated in a future study.

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 (70-200 μm), preparative thin layer chromatography (TLC) on 1 mm SIL G-100 UV_{254} glass plates (Macherey-Nagel) and reaction progress was monitored by TLC on Alugram SIL G UV_{254} (Macherey-Nagel).
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 = 5.84 ppm (^{1}H-NMR) and δ = 49.3 ppm (^{13}C-NMR), CD_3COCD_3: δ = 2.05 ppm (^{1}H-NMR) and δ = 30.8 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, td and q for singlet, doublet, triplet, doublet of doublets, doublet of doublets of doublets, multiplet, doublet of triplets, triplet of doublets and quadruplet, respectively. All coupling constants (*J*) are given in hertz (Hz).
Mass spectra (ESI) were recorded on a TSQ Quantum (Thermo Finnigan) instrument.
Tested compounds have > 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: 4-fluoro-3-methoxybenzoyl chloride;[52] 4-nitro-3-methoxybenzoyl chloride;[52] 3-ethyl-4-methoxybenzeneboronic acid (**20b**);[50] 2-(4-methoxyphenyl)thiophene (**6b**) [53]

General procedure for Friedel-Crafts acylation

Method A: A mixture of monosubstituted thiophene derivative (1 eq), arylcarbonyl chloride (1 eq) and aluminumtrichloride (1 eq) in anhydrous dichloromethane was stirred at 0 °C for 0.5 h. The reaction mixture was warmed to room temperature and stirred for 1 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 procedures for Suzuki coupling

Method B: A mixture of arylbromide (1 eq), boronic acid derivative (1.2 eq), caesium carbonate (4 eq) and tetrakis(triphenylphosphine) palladium (0.01 eq) in an oxygen free DME/water (1:1) solution was refluxed under nitrogen atmosphere for 2h. 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 (except for **17** (CC followed by preparative HPLC) and **19** (CC followed by preparative TLC)).

Method C: A mixture of arylbromide (1 eq), boronic acid derivative (1.2 eq), caesium carbonate (4 eq) and tetrakis(triphenylphosphine) palladium (0.01 eq) was suspended in an oxygen free DME/EtOH/water (1:1:1) solution. The reaction mixture was exposed to microwave irradiation (15 min, 150 W, 150 °C, 15 bar). After reaching room temperature, water was added and the aqueous layer was extracted with ethyl acetate. The combined organic layers were washed with brine, dried over magnesium sulfate, filtered and concentrated to dryness. The product was purified by CC.

General procedures for ether cleavage

Method D: To a solution of methoxybenzene derivative (1 eq) in anhydrous dichloromethane at -78 °C (dry ice/acetone bath), boron tribromide in dichloromethane (1 M, 3 eq per methoxy function) was added dropwise. The reaction mixture was stirred for 20 h 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 sodium sulfate, filtered and concentrated to dryness. The product was purified by CC, CC followed by preparative TLC or CC followed by preparative HPLC, respectively.

Method E: A mixture of methoxybenzene derivative (1eq) and pyridinium hydrochloride (37 eq per methoxy function) was heated to 220 °C for 18 h. After cooling to room temperature, water, 1 M HCl and ethyl acetate were added. The aqueous layer was extracted with ethyl acetate. The combined organic layers were washed with brine, dried over sodium sulfate, filtered and concentrated to dryness. The product was purified by CC followed by preparative TLC or preparative HPLC, respectively.

General procedure for Grignard-reaction

Method F: To a solution of carbaldehyde derivative (1 eq) in anhydrous THF, 3-methoxyphenylmagnesium bromide (1.0 M in THF/toluene, 2.2 eq) was added dropwise. The reaction mixture was stirred for 3 h at 80 °C under nitrogen atmosphere. Brine 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 oxidation

Method G: A mixture of aliphatic alcohol-derivative (1 eq) and 2-iodoxybenzoic acid (2 eq) in anhydrous THF was stirred at 0 °C. After 10 min, the reaction mixture was stirred and heated to 60 °C for 18 h. After cooling to room temperature, saturated sodium thiosulfate solution was added to quench the reaction and the aqueous layer was extracted with ethyl acetate. The

combined organic layers were washed succesively with 1M NaOH and brine, dried over magnesium sulfate, filtered and concentrated to dryness. The product was purified by CC.

General procedure for purification using preparative HPLC

All declared compounds were purified via an Agilent Technologies Series 1200–preparative HPLC using a RP C18 Nucleodur 100-5 column (30 x 100 mm / 50 µM from Macherey Nagel GmbH) as stationary phase with a linear gradient run (solvents: acetonitrile, water) starting from 20 % acetonitrile up to 100 % in 36 min.

Experimental and spectroscopic data of all compounds

2-(3-Methoxyphenyl)thiophene (**1b**). A mixture of 2-bromothiophene (2.00 g, 12.27 mmol), 3-methoxybenzene boronic acid (2.24 g, 14.72 mmol), sodium carbonate (2.73 g, 25.76 mmol) and tetrakis(triphenylphosphine) palladium (142 mg, 123 µmol) in an oxygen free THF/water (1:1) solution was stirred at 100 °C for 18 h under nitrogen atmosphere. The reaction mixture was cooled to rt. 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 (hexane/ethyl acetate 95:5); yield: 96 % (2.24 g). ^{1}H NMR (CD_3COCD_3): 7.45 (dd, *J* = 0.9 Hz and *J* = 3.5 Hz, 1H), 7.44 (dd, *J* = 0.9 Hz and *J* = 5.0 Hz, 1H), 7.32 (t, *J* = 8.2 Hz, 1H), 7.23 (ddd, *J*= 0.9 Hz and *J* = 1.6 Hz and *J* = 7.6 Hz, 1H), 7.21 (t, *J* = 2.1 Hz, 1H), 7.11 (t, *J* = 3.8 Hz, 1H), 6.88 (ddd, *J* = 0.9 Hz and *J* = 2.5 Hz and *J* = 8.2 Hz, 1H), 3.85 (s, 3H); ^{13}C NMR (CD_3COCD_3): 162.20, 145.80, 131.95, 130.00, 126.95, 125.40, 119.95, 114.90, 113.05, 56.60.

(3-Methoxyphenyl)[5-(3-methoxyphenyl)-2-thienyl]methanone (**1a**). The title compound was prepared by reaction of 2-(3-methoxyphenyl)thiophene (**1b**) (100 mg, 0.53 mmol), 3-methoxybenzoyl chloride (90 mg, 0.53 mmol) and aluminum chloride (71 mg, 0.53 mmol) according to method A. The product was purified by CC (hexane/ethyl acetate 97:3); yield: 81 % (138 mg). ^{1}H NMR (CD_3COCD_3): 7.70 (d, *J* = 4.1 Hz, 1H), 7.57 (d, *J* = 4.1 Hz, 1H), 7.49-7.44 (m, 2H), 7.40-7.34 (m, 3H), 7.31 (t, *J* = 2.1 Hz, 1H), 7.21 (ddd, *J* = 1.9 Hz and *J* = 2.5 Hz and *J* = 7.6 Hz, 1H), 6.99 (ddd, *J* = 1.3 Hz and *J* = 2.5 Hz and *J* = 7.9 Hz, 1H), 3.89 (s, 3H), 3.88 (s, 3H); ^{13}C NMR (CD_3COCD_3): 188.65, 162.30, 161.70, 154.30, 144.10, 141.25, 137.85, 136.40, 132.25, 131.50, 126.55, 123.05, 120.40, 120.05, 116.75, 115.50, 113.35, 56.80, 56.75.

(3-Hydroxyphenyl)[5-(3-hydroxyphenyl)-2-thienyl]methanone (**1**). The title compound was prepared by reaction of (3-methoxyphenyl)[5-(3-methoxyphenyl)-2-thienyl]methanone (**1a**) (138 mg, 0.43 mmol) and boron tribromide (2.58 mmol) according to method D. The product

was purified by CC (hexane/ethyl acetate 8:2) followed by preparative HPLC; yield: 75 % (95 mg). ^{1}H NMR (CD_3OD): 7.64 (d, *J* = 4.1 Hz, 1H), 7.42 (d, *J* = 4.1 Hz, 1H), 7.33 (t, *J* = 7.9 Hz, 1H), 7.27 (dt, *J* = 1.3 Hz and *J* = 7.6 Hz, 1H), 7.24-7.17 (m, 3H), 7.12 (t, *J* = 2.1 Hz, 1H), 7.03 (ddd, *J* = 0.9 Hz and *J* = 2.5 Hz and *J* = 7.9 Hz, 1H), 6.80 (ddd, *J* = 0.9 Hz and *J* = 2.5 Hz and *J* = 7.9 Hz, 1H); ^{13}C NMR (CD_3OD): 189.90, 159.40, 159.00, 155.05, 140.60, 139.10, 137.90, 131.45, 130.80, 125.40, 121.30, 120.70, 118.60, 117.50, 116.55, 114.00.

(4-Methoxyphenyl)[5-(3-methoxyphenyl)-2-thienyl]methanone (**2a**). The title compound was prepared by reaction of 2-(3-methoxyphenyl)thiophene (**1b**) (100 mg, 0.53 mmol), 4-methoxybenzoyl chloride (90 mg, 0.53 mmol) and aluminum chloride (71 mg, 0.53 mmol) according to method A. The product was purified by CC (hexane/ethyl acetate 9:1); yield: 96 % (165 mg). ^{1}H NMR (CD_3COCD_3): 7.93 (d, *J* = 9.1 Hz, 2H), 7.70 (d, *J* = 4.1 Hz, 1H), 7.59 (d, *J* = 4.1 Hz, 1H), 7.39 (t, *J* = 7.6 Hz, 1H), 7.36 (td, *J* = 1.3 Hz and *J* = 7.9 Hz, 1H), 7.32 (t, *J* = 2.1 Hz, 1H), 7.10 (d, *J* = 9.1 Hz, 2H), 7.00 (ddd, *J* = 0.9 Hz and *J* = 2.5 Hz and *J* = 7.9 Hz, 1H), 3.92 (s, 3H), 3.89 (s, 3H); ^{13}C NMR (CD_3COCD_3): 187.45, 165.20, 162.35, 153.45, 144.55, 136.95, 136.55, 133.15, 132.25, 126.40, 120.40, 116.60, 115.70, 113.35, 57.00, 56.75.

(4-Hydroxyphenyl)[5-(3-hydroxyphenyl)-2-thienyl]methanone (**2**). The title compound was prepared by reaction of (4-methoxyphenyl)[5-(3-methoxyphenyl)-2-thienyl]methanone (**2a**) (147 mg, 0.45 mmol) and boron tribromide (2.70 mmol) according to method D. The product was purified by CC (hexane/ethyl acetate 8:2) followed by preparative HPLC; yield: 56 % (74 mg). ^{1}H NMR (CD_3COCD_3): 7.87 (d, *J* = 8.8 Hz, 2H), 7.69 (d, *J* = 3.8 Hz, 1H), 7.52 (d, *J* = 3.8 Hz, 1H), 7.31 (t, *J* = 7.6 Hz, 1H), 7.28-7.26 (m, 1H), 7.25-7.24 (m, 1H), 7.01 (d, *J* = 8.8 Hz, 2H), 6.90 (ddd, *J* = 0.9 Hz and *J* = 2.5 Hz and *J* = 7.9 Hz, 1H); ^{13}C NMR (CD_3COCD_3): 187.40, 159.95, 144.50, 136.80, 136.55, 133.45, 132.25, 131.45, 126.00, 119.35, 118.00, 117.15, 114.70.

(2-Methoxyphenyl)[5-(3-methoxyphenyl)-2-thienyl]methanone (**3a**). The title compound was prepared by reaction of 2-(3-methoxyphenyl)thiophene (**1b**) (100 mg, 0.53 mmol), 2-methoxybenzoyl chloride (90 mg, 0.53 mmol) and aluminum chloride (71 mg, 0.53 mmol) according to method A. The product was purified by CC (hexane/ethyl acetate 9:1); yield: 79 % (136 mg). ^{1}H NMR (CD_3COCD_3): 7.55-7.51 (m, 2H), 7.42 (d, *J* = 4.1 Hz, 1H), 7.39 (t, *J* = 7.9 Hz, 2H), 7.35-7.33 (m, 1H), 7.30 (t, *J* = 2.1 Hz, 1H), 7.19 (d, *J* = 8.2 Hz, 1H), 7.10-7.07 (m, 1H), 6.99 (ddd, *J* = 0.9 Hz and *J* = 2.5 Hz and *J* = 8.2 Hz, 1H), 3.88 (s, 3H), 3.81 (s, 3H); ^{13}C NMR (CD_3COCD_3): 189.10, 162.35, 158.85, 154.20, 145.50, 137.70, 136.60, 133.70, 132.25, 130.90, 130.60, 126.55, 122.25, 120.40, 116.75, 113.80, 113.35, 57.05, 56.75.

(2-Hydroxyphenyl)[5-(3-hydroxyphenyl)-2-thienyl]methanone (**3**). The title compound was prepared by reaction of (2-methoxyphenyl)[5-(3-methoxyphenyl)-2-thienyl]methanone (**3a**) (136 mg, 0.42 mmol) and boron tribromide (2.52 mmol) according to method D. The product was purified by CC (hexane/ethyl acetate 8:2) followed by preparative HPLC; yield: 95 % (118 mg). ^{1}H NMR (CD_3COCD_3): 8.03 (dd, J = 1.2 Hz and J = 7.9 Hz, 1H), 7.84 (d, J = 4.3 Hz, 1H), 7.59-7.56 (m, 1H), 7.56 (d, J = 4.3 Hz, 1H), 7.31 (t, J = 7.6 Hz, 1H), 7.29-7.26 (m, 2H), 7.05-7.02 (m, 2H), 6.93 (ddd, J = 1.3 Hz and J = 2.4 Hz and J = 7.9 Hz, 1H); ^{13}C NMR (CD_3COCD_3): 192.00, 163.55, 159.95, 154.80, 142.70, 138.30, 137.60, 136.10, 133.45, 132.35, 126.35, 121.70, 121.05, 119.85, 119.40, 118.35, 114.75.

(5-Bromo-2-thienyl)(3-methoxyphenyl)methanone (**4b**). The title compound was prepared by reaction of 2-bromothiophene (145 mg, 0.89 mmol), 3-methoxybenzoyl chloride (152 mg, 0.89 mmol) and aluminum chloride (119 mg, 0.89 mmol) according to method A. The product was purified by CC (hexane/ethyl acetate 97:3); yield: 77 % (204 mg). ^{1}H NMR (CD_3COCD_3): 7.55 (d, J = 4.1 Hz, 1H), 7.47 (t, J = 7.9 Hz, 1H), 7.42 (td, J = 1.5 Hz and J = 7.9 Hz, 1H), 7.35-7.34 (m, 1H), 7.33 (d, J = 4.1 Hz, 1H), 7.22 (ddd, J = 0.9 Hz and J = 2.5 Hz and J = 8.2 Hz, 1H), 3.88 (s, 3H); ^{13}C NMR (CD_3COCD_3): 187.90, 161.75, 140.45, 137.40, 133.85, 131.60, 123.95, 123.05, 120.45, 115.45, 56.85.

(3-Methoxyphenyl)[5-(4-methoxyphenyl)-2-thienyl]methanone (**4a**). The title compound was prepared by reaction of (5-bromo-2-thienyl)(3-methoxyphenyl)methanone (**4b**) (204 mg, 0.69 mmol), 4-methoxybenzene boronic acid (126 mg, 0.83 mmol), caesium carbonate (899 mg, 2.76 mmol) and tetrakis(triphenylphosphine) palladium (8 mg, 7 µmol) according to method C. The product was purified by CC (hexane/ethyl acetate 95:5); yield: 80 % (178 mg). ^{1}H NMR (CD_3COCD_3): 7.72 (d, J = 8.8 Hz, 2H), 7.66 (d, J = 4.1 Hz, 1H), 7.48-7.43 (m, 3H), 7.38-7.37 (m, 1H), 7.20 (ddd, J = 1.6 Hz and J = 2.5 Hz and J = 7.9 Hz, 1H), 7.02 (d, J = 8.8 Hz, 2H), 3.88 (s, 3H), 3.85 (s, 3H); ^{13}C NMR (CD_3COCD_3): 188.50, 162.65, 161.70, 154.80, 143.05, 141.45, 138.15, 131.45, 129.45, 127.70, 125.15, 123.00, 119.90, 116.50, 115.50, 56.80, 56.75.

(3-Hydroxyphenyl)[5-(4-hydroxyphenyl)-2-thienyl]methanone (**4**). The title compound was prepared by reaction of (3-methoxyphenyl)[5-(4-methoxyphenyl)-2-thienyl]methanone (**4a**) (178 mg, 0.55 mmol) and boron tribromide (3.30 mmol) according to method D. The product was purified by CC (hexane/ethyl acetate 8:2) followed by preparative TLC (hexane/ethyl acetate 1:1); yield: 51 % (83 mg). ^{1}H NMR (CD_3COCD_3): 8.79 (s, 1H), 8.72 (s, 1H), 7.67-7.64 (m, 3H), 7.42 (d, J = 4.1 Hz, 1H), 7.37 (t, J = 7.9 Hz, 1H), 7.35 (td, J = 1.3 Hz and J = 7.6 Hz, 1H), 7.33-7.32 (m, 1H), 7.11 (ddd, J = 0.9 Hz and J = 2.5 Hz and J = 7.9 Hz, 1H), 6.95 (d, J =

8.8 Hz, 1H); ^{13}C NMR (CD_3COCD_3): 188.60, 160.60, 159.35, 155.20, 142.80, 141.50, 138.05, 131.50, 129.60, 126.80, 124.70, 122.00, 121.05, 117.95, 117.25.

(3-Methoxyphenyl)[5-(2-methoxyphenyl)-2-thienyl]methanone (**5a**). The title compound was prepared by reaction of (5-bromo-2-thienyl)(3-methoxyphenyl)methanone (**4b**) (200 mg, 0.67 mmol), 2-methoxybenzene boronic acid (122 mg, 0.80 mmol), caesium carbonate (873 mg, 2.68 mmol) and tetrakis(triphenylphosphine) palladium (8 mg, 7 μmol) according to method C. The product was purified by CC (hexane/ethyl acetate 9:1); yield: 67 % (145 mg). ^{1}H NMR (CD_3COCD_3): 7.85 (dd, *J* = 1.9 Hz and *J* = 7.9 Hz, 2H), 7.69 (d, *J* = 1.3 Hz, 1H), 7.49-7.44 (m, 2H), 7.42-7.38 (m, 2H), 7.21-7.18 (m, 2H), 7.07 (dt, *J* = 0.9 Hz and *J* = 7.9 Hz, 1H), 4.01 (s, 3H), 3.89 (s, 3H); ^{13}C NMR (CD_3COCD_3): 189.10, 161.70, 158.10, 149.85, 144.05, 141.70, 136.50, 132.15, 131.45, 130.15, 127.80, 123.75, 123.10, 122.95, 119.90, 115.50, 114.05, 57.10, 56.80.

(3-Hydroxyphenyl)[5-(2-hydroxyphenyl)-2-thienyl]methanone (**5**). The title compound was prepared by reaction of (3-methoxyphenyl)[5-(2-methoxyphenyl)-2-thienyl]methanone (**5a**) (145 mg, 0.45 mmol) and boron tribromide (2.7 mmol) according to method D. The product was purified by CC (hexane/ethyl acetate 8:2) followed by preparative HPLC; yield: 25 % (33 mg). ^{1}H NMR (CD_3COCD_3): 7.80 (dd, *J* = 1.6 Hz and *J* = 7.9 Hz, 1H), 7.75 (d, *J* = 4.1 Hz, 1H), 7.69 (d, *J* = 4.1 Hz, 1H), 7.39 (t, *J* = 7.6 Hz, 1H), 7.36 (dt, *J* = 1.6 Hz and *J* = 7.6 Hz, 1H), 7.34-7.33 (m, 1H), 7.27-7.23 (m, 1H), 7.11 (ddd, *J* = 1.6 Hz and *J* = 2.5 Hz and *J* = 7.9 Hz, 1H), 7.08 (dd, *J* = 1.3 Hz and *J* = 8.2 Hz, 1H), 6.97 (td, *J* = 1.3 Hz and *J* = 7.9 Hz, 1H); ^{13}C NMR (CD_3COCD_3): 189.10, 159.35, 156.05, 150.55, 143.75, 141.80, 136.55, 131.85, 131.50, 130.20, 127.55, 122.10, 122.05, 120.95, 118.45, 117.30.

(3-Methoxy-4-methylphenyl)[5-(4-methoxyphenyl)-2-thienyl]methanone (**6a**). The title compound was prepared by reaction of 2-(4-methoxyphenyl)thiophene (**6b**) (150 mg, 0.79 mmol), 3-methoxy-4-methylbenzoyl chloride (146 mg, 0.79 mmol) and aluminum chloride (105 mg, 0.79 mmol) according to method A. The product was purified by CC (hexane/ethyl acetate 9:1); yield: 63 % (168 mg). ^{1}H NMR (CD_3COCD_3): 7.73 (d, *J* = 8.8 Hz, 2H), 7.71 (d, *J* = 4.1 Hz, 1H), 7.45 (d, *J* = 4.1 Hz, 1H), 7.41 (dd, *J* = 0.9 Hz and *J* = 7.6 Hz, 1H), 7.38-7.37 (m, 1H), 7.31 (d, *J* = 7.6 Hz, 1H), 7.03 (d, *J* = 8.8 Hz, 2H), 3.93 (s, 3H), 3.86 (s, 3H), 2.27 (s, 3H); ^{13}C NMR (CD_3COCD_3): 188.35, 162.60, 159.70, 154.35, 143.35, 137.80, 133.15, 132.15, 129.45, 127.80, 125.10, 123.25, 116.50, 111.90, 56.85, 56.80, 17.50.

(3-Hydroxy-4-methylphenyl)[5-(4-hydroxyphenyl)-2-thienyl]methanone (**6**). The title compound was prepared by reaction of (3-methoxy-4-methylphenyl)[5-(4-methoxyphenyl)-2-

thienyl]methanone (**6a**) (168 mg, 0.50 mmol) and boron tribromide (3.00 mmol) according to method D. The crude mixture was washed with MeOH. The resulting suspension was filtered and the precipitated product was dried under reduced pressure.; yield: 88 % (137 mg). ^{1}H NMR (CD_3COCD_3): 8.80 (s, 1H), 8.63 (s, 1H), 7.67-7.64 (m, 3H), 7.41 (d, *J* = 4.1 Hz, 1H), 7.36 (d, *J* = 1.6 Hz, 1H), 7.31 (dd, *J* = 1.6 Hz and *J* = 7.6 Hz, 1H), 7.27 (d, *J* = 7.6 Hz, 1H), 6.94 (d, *J* = 8.8 Hz, 2H), 2.29 (s, 3H); ^{13}C NMR (CD_3COCD_3): 188.25, 160.55, 157.25, 154.80, 143.05, 138.95, 137.65, 132.55, 131.35, 129.60, 126.85, 124.60, 122.35, 117.95, 116.65, 17.30.

(4-Fluoro-3-methoxyphenyl)[5-(4-methoxyphenyl)-2-thienyl]methanone (**7a**). The title compound was prepared by reaction of 2-(4-methoxyphenyl)thiophene (**6b**) (150 mg, 0.79 mmol), 4-fluoro-3-methoxybenzoyl chloride (**7b**) (149 mg, 0.79 mmol) and aluminum chloride (105 mg, 0.79 mmol) according to method A. The product was purified by CC (hexane/ethyl acetate 9:1); yield: 88 % (237 mg). ^{1}H NMR (CD_3SOCD_3): 7.76-7.73 (m, 3H), 7.56-7.53 (m, 2H), 7.48-7.45 (m, 1H), 7.41-7.37 (m, 1H), 7.04 (d, *J* = 8.8 Hz, 2H), 3.92 (s, 3H), 3.80 (s, 3H); ^{13}C NMR (CD_3SOCD_3): 185.60, 171.25, 170.10, 163.20, 160.35, 152.70, 147.25, 140.15, 137.25, 134.35, 127.70, 125.20, 124.05, 122.30, 114.75, 114.05, 51.00, 47.90.

(4-Fluoro-3-hydroxyphenyl)[5-(4-hydroxyphenyl)-2-thienyl]methanone (**7**). The title compound was prepared by reaction of (4-fluoro-3-methoxyphenyl)[5-(4-methoxyphenyl)-2-thienyl]methanone (**7a**) (237 mg, 0.69 mmol) and boron tribromide (4.14 mmol) according to method D. The product was purified by CC (hexane/ethyl acetate 8:2) followed by preparative TLC (hexane/ethyl acetate 6:4); yield: 86 % (187 mg). ^{1}H NMR (CD_3COCD_3): 9.23 (s, 1H), 8.93 (s, 1H), 7.66-7.63 (m, 3H), 7.52 (dd, *J* = 2.2 Hz and *J* = 8.5 Hz, 1H), 7.41-7.39 (m, 2H), 7.29-7.25 (m, 1H), 6.93 (d, *J* = 8.8 Hz, 2H); ^{13}C NMR (CD_3COCD_3): 187.40, 160.60, 156.85, 155.25, 154.85, 146.95, 146.85, 142.45, 138.10, 136.75, 129.55, 126.65, 124.70, 123.20, 120.25, 117.95, 117.80.

(3-Methoxy-4-methylphenyl)[5-(3-methoxyphenyl)-2-thienyl]methanone (**8a**). The title compound was prepared by reaction of 2-(3-methoxyphenyl)thiophene (**1b**) (300 mg, 1.58 mmol), 3-methoxy-4-methylbenzoyl chloride (292 mg, 1.58 mmol) and aluminum chloride (211 mg, 1.58 mmol) according to method A. The product was purified by CC (hexane/ethyl acetate 95:5); yield: 58 % (310 mg). ^{1}H NMR (CD_3COCD_3): 7.75 (d, *J* = 4.1 Hz, 1H), 7.60 (d, *J* = 4.1 Hz, 1H), 7.43 (dd, *J* = 1.6 Hz and *J* = 7.6 Hz, 1H), 7.41-7.39 (m, 2H), 7.37 (dt, *J* = 1.3 Hz and *J* = 7.6 Hz, 1H), 7.34-7.32 (m, 2H), 7.01 (ddd, *J* = 1.6 Hz and *J* = 2.5 Hz and *J* = 7.9 Hz, 1H), 3.94 (s, 3H), 3.89 (s, 3H); ^{13}C NMR (CD_3COCD_3): 192.65, 162.35, 132.25, 132.20, 126.55, 123.35, 120.40, 119.75, 113.35, 111.95, 56.90, 17.50.

3-Hydroxy-4-methylphenyl)[5-(3-hydroxyphenyl)-2-thienyl]methanone (**8**). The title compound was prepared by reaction of (3-methoxy-4-methylphenyl)[5-(3-methoxyphenyl)-2-thienyl]methanone (**8a**) (310 mg, 0.92 mmol) and boron tribromide (5.52 mmol) according to method D. The product was purified by CC (hexane/ethyl acetate 8:2); yield: 23 % (65 mg). ^{1}H NMR (CD_3COCD_3): 7.71 (d, *J* = 4.1 Hz, 1H), 7.53 (d, *J* = 4.1 Hz, 1H), 7.38-7.37 (m, 1H), 7.33 (dd, *J* = 1.6 Hz and *J* = 7.6 Hz, 1H), 7.31-7.24 (m, 4H), 6.90 (ddd, *J* = 1.3 Hz and *J* = 2.5 Hz and *J* = 7.9 Hz, 1H), 2.30 (s, 3H); ^{13}C NMR (CD_3COCD_3): 188.35, 159.95, 138.75, 137.35, 136.50, 132.60, 132.30, 129.55, 126.10, 122.40, 119.35, 116.70, 114.75, 114.45, 111.85, 17.35.

(4-Fluoro-3-methoxyphenyl)[5-(3-methoxyphenyl)-2-thienyl]methanone (**9a**). The title compound was prepared by reaction of 2-(3-methoxyphenyl)thiophene (**1b**) (300 mg, 1.58 mmol), 4-fluoro-3-methoxybenzoyl chloride (**7b**) (298 mg, 1.58 mmol) and aluminum chloride (211 mg, 1.58 mmol) according to method A. The product was purified by CC (hexane/ethyl acetate 95:5); yield: 91 % (492 mg). ^{1}H NMR (CD_3COCD_3): 7.76 (d, *J* = 3.9 Hz, 1H), 7.62-7.59 (m, 2H), 7.55-7.52 (m, 1H), 7.40 (t, *J* = 7.8 Hz, 1H), 7.37-7.36 (m, 1H), 7.34-7.30 (m, 2H), 7.01 (ddd, *J* = 1.2 Hz and *J* = 2.4 Hz and *J* = 7.6 Hz, 1H), 4.00 (s, 3H), 3.89 (s, 3H).

(4-Fluoro-3-hydroxyphenyl)[5-(3-hydroxyphenyl)-2-thienyl]methanone (**9**). The title compound was prepared by reaction of (4-fluoro-3-methoxyphenyl)[5-(3-methoxyphenyl)-2-thienyl]methanone (**9a**) (492 mg, 1.44 mmol) and boron tribromide (8.64 mmol) according to method D. The product was purified by CC (hexane/ethyl acetate 85:15) and washed with MeOH. The resulting suspension was filtered and the precipitated product was dried under reduced pressure.; yield: 50 % (225 mg). ^{1}H NMR (CD_3SOCD_3): 7.69 (d, *J* = 4.1 Hz, 1H), 7.58 (d, *J* = 4.1 Hz, 1H), 7.44-7.42 (m, 1H), 7.33-7.31 (m, 2H), 7.27 (t, *J* = 7.9 Hz, 1H), 7.22-7.21 (m, 1H), 7.15-7.14 (m, 1H), 6.84-6.82 (m, 1H); ^{13}C NMR (CD_3SOCD_3): 185.65, 158.00, 154.75, 152.80, 152.25, 145.25, 141.00, 136.55, 133.70, 130.55, 124.95, 120.85, 118.15, 116.95, 116.55, 116.30, 112.65.

(3-Methoxy-4-nitrophenyl)[5-(3-methoxyphenyl)-2-thienyl]methanone (**10a**). The title compound was prepared by reaction of 2-(3-methoxyphenyl)thiophene (**1b**) (200 mg, 1.05 mmol), 3-methoxy-4-nitrobenzoyl chloride (**10b**) (226 mg, 1.05 mmol) and aluminum chloride (140 mg, 1.05 mmol) according to method A. The product was purified by CC (hexane/ethyl acetate 9:1) followed by preparative HPLC; yield: 41 % (160 mg). ^{1}H NMR (CD_3COCD_3): 7.98 (d, *J* = 8.2 Hz, 1H), 7.80 (d, *J* = 4.3 Hz, 1H), 7.71 (d, *J* = 1.5 Hz, 1H), 7.63 (d, *J* = 4.0 Hz, 1H), 7.57 (dd, *J* = 1.5 Hz and *J* = 8.2 Hz, 1H), 7.41 (t, *J* = 7.8 Hz, 1H), 7.37 (dt, *J* = 1.5 Hz and *J* = 7.6 Hz, 1H), 7.33 (t, *J* = 1.9 Hz, 1H), 7.02 (ddd, *J* = 1.5 Hz and *J* = 2.4 Hz and *J* = 7.9 Hz, 1H), 4.09 (s, 3H), 3.89 (s, 3H); ^{13}C NMR (CD_3COCD_3): 187.45, 162.30, 155.45, 154.05, 144.45,

143.25, 139.00, 136.15, 132.30, 126.90, 126.80, 122.50, 120.50, 117.00, 115.95, 113.40, 58.35, 56.75.

3-Hydroxy-4-nitrophenyl)[5-(3-hydroxyphenyl)-2-thienyl]methanone (**10**). The title compound was prepared by reaction of (3-methoxy-4-nitrophenyl)[5-(3-methoxyphenyl)-2-thienyl]methanone (**10a**) (160 mg, 0.43 mmol) and boron tribromide (2.58 mmol) according to method D. The product was purified by CC (hexane/ethyl acetate 8:2) followed by preparative HPLC; yield: 29 % (42 mg). ^{1}H NMR (CD_3COCD_3): 8.25 (d, *J* = 8.5 Hz, 1H), 7.75 (d, *J* = 4.1 Hz, 1H), 7.59 (d, *J* = 1.9 Hz, 1H), 7.57 (d, *J* = 4.1 Hz, 1H), 7.46 (dd, *J* = 1.9 Hz and *J* = 8.5 Hz, 1H), 7.32 (t, *J* = 7.8 Hz, 1H), 7.28 (dt, *J* = 1.5 Hz and *J* = 7.9 Hz, 1H), 7.25 (t, *J* = 2.1 Hz, 1H), 6.93 (ddd, *J* = 1.3 Hz and *J* = 2.2 Hz and *J* = 7.6 Hz, 1H); ^{13}C NMR (CD_3COCD_3): 187.30, 160.00, 156.15, 155.90, 146.95, 142.90, 139.00, 132.40, 127.55, 126.65, 122.10, 121.30, 119.50, 118.50, 114.85.

[5-(3-Methoxy-4-methylphenyl)-2-thienyl](3-methoxyphenyl)methanone (**11a**). The title compound was prepared by reaction of (5-bromo-2-thienyl)(3-methoxyphenyl)methanone (**4b**) (200 mg, 0.67 mmol), 3-methoxy-4-methylbenzene boronic acid (133 mg, 0.80 mmol), caesium carbonate (873 mg, 2.68 mmol) and tetrakis(triphenylphosphine) palladium (8 mg, 7 µmol) according to method C. The product was purified by CC (hexane/ethyl acetate 9:1); yield: 45 % (102 mg). ^{1}H NMR (CD_3COCD_3): 7.70 (d, *J* = 4.1 Hz, 1H), 7.56 (d, *J* = 4.1 Hz, 1H), 7.48 (t, *J* = 7.6 Hz, 1H), 7.45 (dt, *J* = 1.6 Hz and *J* = 7.6 Hz, 1H), 7.38-7.37 (m, 1H), 7.30-7.27 (m, 2H), 7.23-7.20 (m, 2H), 3.95 (s, 3H), 3.89 (s, 3H), 2.21 (s, 3H); ^{13}C NMR (CD_3COCD_3): 188.65, 161.70, 160.25, 155.00, 143.55, 141.35, 137.95, 134.10, 133.00, 131.50, 129.75, 126.00, 123.05, 120.10, 120.00, 115.50, 109.70, 56.90, 56.80, 17.25.

5-(3-Hydroxy-4-methylphenyl)-2-thienyl](3-hydroxyphenyl)methanone (**11**). The title compound was prepared by reaction of [5-(3-methoxy-4-methylphenyl)-2-thienyl](3-methoxyphenyl)methanone (**11a**) (90 mg, 0.27 mmol) and boron tribromide (1.62 mmol) according to method D. The product was purified by CC (hexane/ethyl acetate 7:3) followed by preparative HPLC; yield: 49 % (41 mg). ^{1}H NMR (CD_3COCD_3): 7.68 (d, *J* = 4.1 Hz, 1H), 7.46 (d, *J* = 4.1 Hz, 1H), 7.40 (t, *J* = 7.6 Hz, 1H), 7.35 (dt, *J* = 1.6 Hz and *J* = 7.6 Hz, 1H), 7.33-7.32 (m, 1H), 7.25-7.24 (m, 1H), 7.20 (d, *J* = 1.3 Hz, 1H), 7.12 (ddd, *J* = 1.3 Hz and *J* = 2.5 Hz and *J* = 7.9 Hz, 1H), 2.24 (s, 3H); ^{13}C NMR (CD_3COCD_3): 187.55, 157.80, 154.80, 137.85, 133.85, 133.45, 131.55, 127.95, 125.60, 124.60, 122.00, 121.15, 119.30, 118.40, 117.25, 114.05, 108.95, 17.05.

[5-(4-Fluoro-3-methoxyphenyl)-2-thienyl](3-methoxyphenyl)methanone (**12a**). The title compound was prepared by reaction of (5-bromo-2-thienyl)(3-methoxyphenyl)methanone (**4b**) (200 mg, 0.67 mmol), 4-fluoro-3-methoxybenzene boronic acid (136 mg, 0.80 mmol), caesium carbonate (873 mg, 2.68 mmol) and tetrakis(triphenylphosphine) palladium (8 mg, 7 µmol) according to method C. The product was purified by CC (hexane/ethyl acetate 9:1); yield: 69 % (159 mg). ^{1}H NMR (CD_3COCD_3): 7.70 (d, J = 4.1 Hz, 1H), 7.56 (d, J = 4.1 Hz, 1H), 7.51 (dd, J = 2.2 Hz and J = 8.2 Hz, 1H), 7.48 (t, J = 7.6 Hz, 1H), 7.45 (td, J = 1.6 Hz and J = 7.6 Hz, 1H), 7.38-7.34 (m, 2H), 7.25-7.20 (m, 2H), 4.00 (s, 3H), 3.89 (s, 3H); ^{13}C NMR (CD_3COCD_3): 188.65, 161.75, 155.75, 153.75, 150.25, 144.20, 141.25, 137.90, 132.10, 131.50, 126.65, 123.05, 120.85, 120.10, 118.50, 115.50, 113.55, 57.75, 56.85.

[5-(4-Fluoro-3-hydroxyphenyl)-2-thienyl](3-hydroxyphenyl)methanone (**12**). The title compound was prepared by reaction of [5-(4-fluoro-3-methoxyphenyl)-2-thienyl](3-methoxyphenyl)methanone (**12a**) (159 mg, 0.46 mmol) and boron tribromide (2.76 mmol) according to method D. The product was purified by CC (hexane/ethyl acetate 7:3) followed by preparative HPLC; yield: 84 % (121 mg). ^{1}H NMR (CD_3COCD_3): 8.85 (s, 1H), 7.68 (d, J = 4.1 Hz, 1H), 7.48 (d, J = 4.1 Hz, 1H), 7.42-7.33 (m, 4H), 7.29-7.26 (m, 1H), 7.22-7.18 (m, 1H), 7.13 (ddd, J = 1.3 Hz and J = 2.5 Hz and J = 7.6 Hz, 1H); ^{13}C NMR (CD_3COCD_3): 188.70, 159.40, 155.00, 153.40, 147.40, 144.15, 141.30, 137.80, 132.10, 131.55, 126.30, 122.10, 121.25, 119.95, 118.75, 117.30.

[5-(2-Fluoro-3-methoxyphenyl)-2-thienyl](3-methoxyphenyl)methanone (**13a**). The title compound was prepared by reaction of (5-bromo-2-thienyl)(3-methoxyphenyl)methanone (**4b**) (200 mg, 0.67 mmol), 2-fluoro-3-methoxybenzene boronic acid (136 mg, 0.80 mmol), caesium carbonate (873 mg, 2.68 mmol) and tetrakis(triphenylphosphine) palladium (8 mg, 7 µmol) according to method C. The product was purified by CC (hexane/ethyl acetate 9:1); yield: 33 % (76 mg). ^{1}H NMR ($CDCl_3$): 7.66 (d, J = 4.0 Hz, 1H), 7.51 (dd, J = 1.2 Hz and J = 4.0 Hz, 1H), 7.47 (dt, J = 1.2 Hz and J = 7.6 Hz, 1H), 7.42-7.39 (m, 2H), 7.25 (td, J = 1.5 Hz and J = 7.9 Hz, 1H), 7.14-7.11 (m, 2H), 6.98 (dt, J = 1.5 Hz and J = 8.2 Hz, 1H), 3.93 (s, 3H), 3.87 (s, 3H); ^{13}C NMR ($CDCl_3$): 187.80, 159.60, 150.45, 148.45, 145.75, 142.75, 139.25, 135.35, 129.40, 127.35, 124.35, 124.30, 122.00, 121.90, 120.20, 118.65, 113.65, 56.40, 55.45.

[5-(2-Fluoro-3-hydroxyphenyl)-2-thienyl](3-hydroxyphenyl)methanone (**13**). The title compound was prepared by reaction of [5-(2-fluoro-3-methoxyphenyl)-2-thienyl](3-methoxyphenyl)methanone (**13a**) (76 mg, 0.22 mmol) and boron tribromide (1.32 mmol) according to method D. The product was purified by CC (hexane/ethyl acetate 7:3) followed by preparative HPLC; yield: 77 % (53 mg). ^{1}H NMR (CD_3COCD_3): 8.89 (s, 1H), 7.74 (dd, J = 1.3

Hz and *J* = 4.1 Hz, 1H), 7.63 (dd, *J* = 0.9 Hz and *J* = 4.0 Hz, 1H), 7.42-7.37 (m, 2H), 7.36.7.35 (m, 1H), 7.31-7.28 (m, 1H), 7.15-7.11 (m, 2H), 7.07 (td, *J* = 1.9 Hz and *J* = 8.2 Hz, 1H); ^{13}C NMR (CD_3COCD_3): 188.90, 159.35, 151.40, 149.45, 147.80, 147.05, 144.90, 141.25, 137.00, 131.60, 129.20, 126.80, 123.70, 122.10, 121.30, 120.15, 117.30.

[5-(4-Methoxy-3-methylphenyl)-2-thienyl](3-methoxyphenyl)methanone (**14a**). The title compound was prepared by reaction of (5-bromo-2-thienyl)(3-methoxyphenyl)methanone (**4b**) (200 mg, 0.67 mmol), 4-methoxy-3-methylbenzene boronic acid (133 mg, 0.80 mmol), caesium carbonate (873 mg, 2.68 mmol) and tetrakis(triphenylphosphine) palladium (8 mg, 7 μmol) according to method B. The product was purified by CC (hexane/ethyl acetate 9:1); yield: 79 % (180 mg). ^{1}H NMR (CD_3COCD_3): 7.68 (d, *J* = 4.1 Hz, 1H), 7.64-7.60 (m, 2H), 7.48 (t, *J* = 7.8 Hz, 1H), 7.46-7.43 (m, 2H), 7.37-7.36 (m, 1H), 7.21 (ddd, *J* = 1.3 Hz and *J* = 2.5 Hz and *J* = 7.9 Hz, 1H), 7.02 (d, *J* = 8.2 Hz, 1H), 3.90 (s, 3H), 3.89 (s, 3H), 2.24 (s, 3H); ^{13}C NMR (CD_3COCD_3): 188.55, 161.70, 160.85, 155.20, 142.90, 141.50, 138.20, 131.45, 130.25, 129.10, 127.35, 127.05, 125.00, 123.00, 119.90, 115.45, 112.50, 56.95, 56.80, 17.25.

[5-(4-Hydroxy-3-methylphenyl)-2-thienyl](3-hydroxyphenyl)methanone (**14**). The title compound was prepared by reaction of [5-(4-methoxy-3-methylphenyl)-2-thienyl](3-methoxyphenyl)methanone (**14a**) (180 mg, 0.53 mmol) and boron tribromide (3.18 mmol) according to method D. The product was purified by CC (hexane/ethyl acetate 7:3); yield: 90 % (148 mg). ^{1}H NMR (CD_3COCD_3): 8.68 (s, 1H), 8.66 (s, 1H), 7.65 (d, *J* = 4.1 Hz, 1H), 7.57-7.56 (m, 1H), 7.47 (dd, *J* = 2.2 Hz and *J* = 8.2 Hz, 1H), 7.41 (d, *J* = 4.1 Hz, 1H), 7.39 (t, *J* = 7.8 Hz, 1H), 7.35 (dt, *J* = 1.3 Hz and *J* = 7.6 Hz, 1H), 7.32-7.31 (m, 1H), 7.11 (ddd, *J* = 1.3 Hz and *J* = 2.5 Hz and *J* = 7.9 Hz, 1H), 6.92 (d, *J* = 8.2 Hz, 1H), 2.27 (s, 3H); ^{13}C NMR (CD_3COCD_3): 188.55, 159.35, 158.65, 155.50, 142.65, 141.60, 138.05, 131.50, 130.70, 127.25, 126.90, 126.75, 124.55, 122.00, 121.00, 117.25, 117.20, 17.10.

[5-(3-Fluoro-4-methoxyphenyl)-2-thienyl](3-methoxyphenyl)methanone (**15a**). The title compound was prepared by reaction of (5-bromo-2-thienyl)(3-methoxyphenyl)methanone (**4b**) (200 mg, 0.67 mmol), 3-fluoro-4-methoxybenzene boronic acid (136 mg, 0.80 mmol), caesium carbonate (873 mg, 2.68 mmol) and tetrakis(triphenylphosphine) palladium (8 mg, 7 μmol) according to method B. The product was purified by CC (hexane/ethyl acetate 9:1); yield: 93 % (213 mg). ^{1}H NMR (CD_3COCD_3): 7.70 (d, *J* = 4.1 Hz, 1H), 7.61-7.56 (m, 2H), 7.53 (d, *J* = 4.1 Hz, 1H), 7.48 (t, *J* = 7.8 Hz, 1H), 7.45 (dt, *J* = 1.3 Hz and *J* = 7.6 Hz, 1H), 7.38-7.37 (m, 1H), 7.26-7.20 (m, 2H), 3.95 (s, 3H), 3.89 (s, 3H); ^{13}C NMR (CD_3COCD_3): 188.60, 161.75, 155.20, 153.25, 150.50, 143.75, 141.30, 138.05, 131.50, 128.20, 126.05, 124.55, 123.05, 120.10, 116.10, 115.60, 115.45, 57.70, 56.85.

[5-(3-Fluoro-4-hydroxyphenyl)-2-thienyl](3-hydroxyphenyl)methanone (**15**). The title compound was prepared by reaction of [5-(3-fluoro-4-methoxyphenyl)-2-thienyl](3-methoxyphenyl)methanone (**15a**) (200 mg, 0.58 mmol) and boron tribromide (3.48 mmol) according to method D. The product was purified by CC (hexane/ethyl acetate 7:3) followed by preparative TLC (hexane/ethyl acetate 6:4); yield: 90 % (164 mg). ^{1}H NMR (CD_3COCD_3): 9.09 (s, 1H), 8.74 (s, 1H), 7.66 (d, *J* = 4.1 Hz, 1H), 7.56 (dd, *J* = 2.2 Hz and *J* = 12.3 Hz, 1H), 7.47-7.44 (m, 2H), 7.39 (t, *J* = 7.8 Hz, 1H), 7.35 (dt, *J* = 1.5 Hz and *J* = 7.6 Hz, 1H), 7.34-7.32 (m, 1H), 7.13 (ddd, *J* = 1.3 Hz and *J* = 2.5 Hz and *J* = 7.8 Hz, 1H), 7.09 (t, *J* = 8.8 Hz, 1H); ^{13}C NMR (CD_3COCD_3): 188.65, 159.35, 154.45, 153.45, 147.80, 143.50, 141.30, 137.95, 131.55, 127.50, 125.65, 124.70, 122.05, 121.15, 120.35, 117.25, 115.60.

(3-Methoxyphenyl){5-[4-methoxy-3-(trifluoromethyl)phenyl]-2-thienyl}methanone (**16a**). The title compound was prepared by reaction of (5-bromo-2-thienyl)(3-methoxyphenyl)methanone (**4b**) (150 mg, 0.51 mmol), 4-methoxy-3-trifluoromethylbenzene boronic acid (134 mg, 0.61 mmol), caesium carbonate (665 mg, 2.04 mmol) and tetrakis(triphenylphosphine) palladium (6 mg, 5 μmol) according to method B. The product was purified by CC (hexane/ethyl acetate 8:2); yield: 98 % (196 mg). ^{1}H NMR (CD_3COCD_3): 8.04 (ddd, *J* = 0.6 Hz and *J* = 2.5 Hz and *J* = 8.5 Hz, 1H), 8.00 (d, *J* = 2.2 Hz, 1H), 7.73 (d, *J* = 4.1 Hz, 1H), 7.62 (d, *J* = 4.1 Hz, 1H), 7.49 (t, *J* = 7.9 Hz, 1H), 7.46 (dt, *J* = 1.5 Hz and *J* = 7.6 Hz, 1H), 7.39-7.37 (m, 2H), 7.22 (ddd, *J* = 1.3 Hz and *J* = 2.5 Hz and *J* = 7.9 Hz, 1H), 4.02 (s, 3H), 3.90 (s, 3H); ^{13}C NMR (CD_3COCD_3): 188.60, 161.75, 152.70, 144.05, 141.25, 138.10, 133.60, 131.55, 127.55, 126.40, 123.05, 120.15, 115.50, 115.45, 57.85, 56.85.

(3-Hydroxyphenyl){5-[4-hydroxy-3-(trifluoromethyl)phenyl]-2-thienyl}methanone (**16**). The title compound was prepared by reaction of (3-methoxyphenyl){5-[4-methoxy-3-(trifluoromethyl)phenyl]-2-thienyl}methanone (**16a**) (196 mg, 0.50 mmol) and boron tribromide (3.00 mmol) according to method D. The product was purified by CC (hexane/ethyl acetate 6:4) followed by preparative HPLC; yield: 42 % (77 mg). ^{1}H NMR (CD_3COCD_3): 7.94 (d, *J* = 2.2 Hz, 1H), 7.88 (ddd, *J* = 0.6 Hz and *J* = 2.5 Hz and *J* = 8.5 Hz, 1H), 7.70 (d, *J* = 4.1 Hz, 1H), 7.56 (d, *J* = 4.1 Hz, 1H), 7.40 (td, *J* = 0.6 Hz and *J* = 7.6 Hz, 1H), 7.36 (dt, *J* = 1.5 Hz and *J* = 7.6 Hz, 1H), 7.34-7.33 (m, 1H), 7.23 (d, *J* = 8.5 Hz, 1H), 7.13 (ddd, *J* = 1.3 Hz and *J* = 2.5 Hz and *J* = 7.9 Hz, 1H); ^{13}C NMR (CD_3COCD_3): 188.65, 159.40, 158.35, 153.10, 143.80, 141.30, 137.95, 133.30, 131.55, 126.75, 126.45, 125.90, 122.05, 121.20, 119.90, 119.25, 117.25.

(5-Bromo-2-thienyl)(3-hydroxyphenyl)methanone (**17a**). The title compound was prepared by reaction of (5-bromo-2-thienyl)(3-methoxyphenyl)methanone (**4b**) (650 mg, 2.19 mmol) and boron tribromide (6.57 mmol) according to method D. The product was used in the next step

without purification; yield: 100 % (620 mg). ^{1}H NMR (CD_3COCD_3): 8.76 (s, 1H), 7.54 (d, *J* = 4.1 Hz, 1H), 7.39 (t, *J* = 7.8 Hz, 1H), 7.34-7.32 (m, 2H), 7.30 (t, *J* = 2.1 Hz, 1H), 7.13 (ddd, *J* = 1.3 Hz and *J* = 2.5 Hz and *J* = 8.2 Hz, 1H); ^{13}C NMR (CD_3COCD_3): 187.90, 159.45, 147.25, 140.45, 137.25, 133.80, 131.70, 123.80, 122.10, 121.60, 117.25.

[5-(3-Chloro-4-hydroxyphenyl)-2-thienyl](3-hydroxyphenyl)methanone (**17**). The title compound was prepared by reaction of (5-bromo-2-thienyl)(3-hydroxyphenyl)methanone (**17a**) (150 mg, 0.53 mmol), 3-chloro-4-hydroxybenzene boronic acid (110 mg, 0.64 mmol), caesium carbonate (691 mg, 2.12 mmol) and tetrakis(triphenylphosphine) palladium (6 mg, 5 µmol) according to method B. The product was purified by CC (hexane/ethyl acetate 8:2) followed by preparative HPLC; yield: 42 % (73 mg). ^{1}H NMR (CD_3COCD_3): 7.79 (d, *J* = 2.2 Hz, 1H), 7.68 (d, *J* = 4.1 Hz, 1H), 7.60 (dd, *J* = 2.2 Hz and *J* = 8.5 Hz, 1H), 7.51 (d, *J* = 4.1 Hz, 1H), 7.39 (td, *J* = 0.6 Hz and *J* = 7.8 Hz, 1H), 7.35 (dt, *J* = 1.5 Hz and *J* = 7.8 Hz, 1H), 7.33-7.32 (m, 1H), 7.13-7.11 (m, 2H); ^{13}C NMR (CD_3COCD_3): 188.60, 159.40, 156.05, 153.20, 143.55, 141.35, 137.95, 131.55, 129.40, 128.10, 128.00, 125.65, 123.10, 122.00, 121.15, 119.30, 117.25.

[5-(3,4-Dimethoxyphenyl)-2-thienyl](3-methoxyphenyl)methanone (**18a**). The title compound was prepared by reaction of (5-bromo-2-thienyl)(3-methoxyphenyl)methanone (**4b**) (200 mg, 0.67 mmol), 3,4-dimethoxybenzene boronic acid (146 mg, 0.80 mmol), caesium carbonate (873 mg, 2.68 mmol) and tetrakis(triphenylphosphine) palladium (8 mg, 7 µmol) according to method C. The product was purified by CC (hexane/ethyl acetate 8:2); yield: 97 % (230 mg). ^{1}H NMR (CD_3COCD_3): 7.67 (d, *J* = 4.1 Hz, 1H), 7.48-7.43 (m, 3H), 7.37-7.36 (m, 1H), 7.35-7.33 (m, 2H), 7.20 (ddd, *J* = 1.3 Hz and *J* = 2.5 Hz and *J* = 7.9 Hz, 1H), 7.03 (d, *J* = 8.8 Hz, 1H), 3.91 (s, 3H), 3.88 (s, 3H), 3.86 (s, 3H); ^{13}C NMR (CD_3COCD_3): 188.55, 161.75, 152.70, 151.85, 143.10, 141.50, 138.05, 131.45, 128.05, 125.40, 123.00, 121.00, 119.95, 115.50, 114.15, 111.85, 57.35, 57.25, 56.85.

[5-(3,4-Dihydroxyphenyl)-2-thienyl](3-hydroxyphenyl)methanone (**18**). The title compound was prepared by reaction of [5-(3,4-dimethoxyphenyl)-2-thienyl](3-methoxyphenyl)methanone (**18a**) (230 mg, 0.65 mmol) and boron tribromide (5.85 mmol) according to method D. The product was purified by CC (hexane/ethyl acetate 6:4) followed by preparative HPLC; yield: 28 % (56 mg). ^{1}H NMR (CD_3COCD_3): 8.47 (s, 1H), 8.45 (s, 1H), 7.64 (d, *J* = 4.1 Hz, 1H), 7.40-7.37 (m, 2H), 7.33 (dt, *J* = 1.6 Hz and *J* = 7.6 Hz, 1H), 7.32-7.31 (m, 1H), 7.27 (d, *J* = 2.2 Hz, 1H), 7.17 (dd, *J* = 1.9 Hz and *J* = 8.2 Hz, 1H), 7.11 (ddd, *J* = 0.9 Hz and *J* = 2.5 Hz and *J* = 7.9 Hz, 1H), 6.92, (d, *J* = 8.2 Hz, 1H); ^{13}C NMR (CD_3COCD_3): 188.60, 159.35, 155.35, 148.60, 147.50, 142.75, 141.50, 138.05, 131.50, 127.30, 124.75, 122.00, 121.00, 120.30, 117.80, 117.20, 115.05.

[5-(4-Hydroxy-3-methoxyphenyl)-2-thienyl](3-hydroxyphenyl)methanone (**19**). The title compound was prepared by reaction of (5-bromo-2-thienyl)(3-hydroxyphenyl)methanone (**17a**) (150 mg, 0.53 mmol), 4-hydroxy-3-methoxybenzene boronic acid pinacol ester (160 mg, 0.64 mmol), caesium carbonate (691 mg, 2.12 mmol) and tetrakis(triphenylphosphine) palladium (6 mg, 5 μmol) according to method B. The product was purified by CC (hexane/ethyl acetate 8:2) followed by preparative TLC (hexane/ethyl acetate 6:4); yield: 73 % (126 mg). ^{1}H NMR (CD_3COCD_3): 7.66 (d, *J* = 4.1 Hz, 1H), 7.47 (d, *J* = 4.1 Hz, 1H), 7.39 (t, *J* = 7.8 Hz, 1H), 7.36-7.34 (m, 2H), 7.32-7.31 (m, 1H), 7.29 (dd, *J* = 2.2 Hz and *J* = 8.2 Hz, 1H), 7.11 (ddd, *J* = 0.9 Hz and *J* = 2.5 Hz and *J* = 7.8 Hz, 1H), 6.93 (d, *J* = 8.2 Hz, 1H), 3.96 (s, 3H); ^{13}C NMR (CD_3COCD_3): 188.60, 159.40, 155.35, 149.95, 142.90, 141.55, 137.95, 131.50, 127.15, 125.00, 122.00, 121.50, 121.05, 117.55, 117.25, 111.55, 57.50.

[5-(3-Ethyl-4-methoxyphenyl)-2-thienyl](3-hydroxyphenyl)methanone (**20a**). The title compound was prepared by reaction of (5-bromo-2-thienyl)(3-hydroxyphenyl)methanone (**17a**) (200 mg, 0.71 mmol), 3-ethyl-4-methoxybenzene boronic acid (153 mg, 0.85 mmol), caesium carbonate (925 mg, 2.84 mmol) and tetrakis(triphenylphosphine) palladium (8 mg, 7 μmol) according to method B. The product was purified by CC (hexane/ethyl acetate 9:1); yield: 65 % (155 mg). ^{1}H NMR (CD_3COCD_3): 8.71 (s, 1H), 7.67 (d, *J* = 4.1 Hz, 1H), 7.62 (dd, *J* = 2.5 Hz and *J* = 8.2 Hz, 1H), 7.60-7.59 (m, 1H), 7.47 (d, *J* = 4.1 Hz, 1H), 7.40 (td, *J* = 0.6 Hz and *J* = 7.9 Hz, 1H), 7.35 (dt, *J* = 1.5 Hz and *J* = 7.6 Hz, 1H), 7.33-7.32 (m, 1H), 7.12 (ddd, *J* = 1.3 Hz and *J* = 2.5 Hz and *J* = 7.9 Hz, 1H), 7.05 (d, *J* = 8.2 Hz, 1H), 3.91 (s, 3H), 2.68 (q, *J* = 7.6 Hz, 2H), 1.21 (t, *J* = 7.6 Hz, 3H); ^{13}C NMR (CD_3COCD_3): 188.60, 159.35, 138.00, 131.55, 128.80, 127.55, 127.05, 125.00, 122.00, 121.00, 119.95, 117.20, 112.85, 56.95, 24.95, 15.55.

[5-(3-Ethyl-4-hydroxyphenyl)-2-thienyl](3-hydroxyphenyl)methanone (**20**). The title compound was prepared by reaction of [5-(3-ethyl-4-methoxyphenyl)-2-thienyl](3-hydroxyphenyl)methanone (**20a**) (116 mg, 0.34 mmol) and pyridinium hydrochloride (1.45 g, 12.58 mmol) according to method E. The product was purified by CC (hexane/ethyl acetate 6:4) followed by preparative TLC (hexane/ethyl acetate 6:4); yield: 47 % (52 mg). ^{1}H NMR (CD_3COCD_3): 8.72 (s, 1H), 8.68 (s, 1H), 7.66 (d, *J* = 4.1 Hz, 1H), 7.57 (d, *J* = 2.5 Hz, 1H), 7.47 (d, *J* = 2.5 Hz and *J* = 8.2 Hz, 1H), 7.42 (d, *J* = 4.1 Hz, 1H), 7.39 (t, *J* = 7.9 Hz, 1H), 7.34 (dt, *J* = 1.5 Hz and *J* = 7.6 Hz, 1H), 7.32-7.31 (m, 1H), 7.11 (ddd, *J* = 1.3 Hz and *J* = 2.5 Hz and *J* = 7.9 Hz, 1H), 6.93 (d, *J* = 8.2 Hz, 1H), 2.70 (q, *J* = 7.6 Hz, 2H), 1.24 (t, *J* = 7.6 Hz, 3H); ^{13}C NMR (CD_3COCD_3): 187.60, 158.35, 157.30, 154.65, 149.55, 141.65, 140.60, 137.05, 132.35, 130.50, 128.25, 125.90, 123.60, 121.00, 120.00, 116.50, 116.25, 23.90, 14.50.

(4-Bromo-2-thienyl)(3-methoxyphenyl)methanol (**21c**). The title compound was prepared by reaction of 4-bromothiophene-2-carbaldehyde (450 mg, 2.35 mmol) and 3-methoxyphenylmagnesium bromide (5.17 mmol) according to method F. The product was purified by CC (hexane/ethyl acetate 9:1); yield: 90 % (630 mg). ^{1}H NMR (CD_3COCD_3): 7.32 (d, *J* = 0.9 Hz, 1H), 7.28 (t, *J* = 7.9 Hz, 1H), 7.13 (t, *J* = 2.2 Hz, 1H), 7.08-7.06 (m, 1H), 6.90 (t, *J* = 1.3 Hz, 1H), 6.87 (ddd, *J* = 0.9 Hz and *J* = 2.5 Hz and *J* = 8.2 Hz, 1H), 6.05 (s, 1H), 5.42 (s, 1H), 3.77 (s, 3H); ^{13}C NMR (CD_3COCD_3): 161.45, 153.15, 147.00, 131.10, 128.10, 123.95, 120.15, 114.65, 113.50, 110.00, 72.85, 56.35.

(4-Bromo-2-thienyl)(3-methoxyphenyl)methanone (**21b**). The title compound was prepared by reaction of (4-bromo-2-thienyl)(3-methoxyphenyl)methanol (**21c**) (630 mg, 2.11 mmol) and 2-iodoxybenzoic acid (1.19 g, 4.22 mmol) according to method G. The product was purified by CC (hexane/ethyl acetate 9:1); yield: 79 % (498 mg). ^{1}H NMR (CD_3COCD_3): 7.95 (d, *J* = 1.5 Hz, 1H), 7.65 (d, *J* = 1.5 Hz, 1H), 7.48-7.44 (m, 2H), 7.38-7.37 (m, 1H), 7.22-7.19 (m, 1H), 3.87 (s, 3H); ^{13}C NMR (CD_3COCD_3): 187.70, 161.65, 145.75, 140.25, 138.00, 133.80, 131.55, 123.20, 120.50, 115.50, 111.95, 56.80.

[4-(4-Methoxy-3-methylphenyl)-2-thienyl](3-methoxyphenyl)methanone (**21a**). The title compound was prepared by reaction of (4-bromo-2-thienyl)(3-methoxyphenyl)methanone (**21b**) (200 mg, 0.67 mmol), 4-methoxy-3-methylbenzeneboronic acid (133 mg, 0.80 mmol), caesium carbonate (873 mg, 2.68 mmol) and tetrakis(triphenylphosphine) palladium (8 mg, 7 μmol) according to method B. The product was purified by CC (hexane/ethyl acetate 9:1); yield: 71 % (160 mg). ^{1}H NMR (CD_3COCD_3): 8.00 (d, *J* = 1.5 Hz, 1H), 7.97 (d, *J* = 1.5 Hz, 1H), 7.51-7.46 (m, 4H), 7.43-7.42 (m, 1H), 7.20 (ddd, *J* = 0.9 Hz and *J* = 2.5 Hz and *J* = 8.2 Hz, 1H), 6.90 (d, *J* = 8.2 Hz; 1H), 3.87 (s, 3H), 3.83 (s, 3H), 2.20 (s, 3H); ^{13}C NMR (CD_3COCD_3): 188.85, 161.65, 159.45, 145.55, 144.95, 141.25, 134.80, 131.45, 130.35, 129.30, 128.75, 128.45, 126.75, 123.25, 120.10, 115.55, 112.15, 56.75, 56.70, 17.40.

[4-(4-Hydroxy-3-methylphenyl)-2-thienyl](3-hydroxyphenyl)methanone (**21**). The title compound was prepared by reaction of [4-(4-methoxy-3-methylphenyl)-2-thienyl](3-methoxyphenyl)methanone (**21a**) (160 mg, 0.47 mmol) and boron tribromide (2.82 mmol) according to method D. The product was purified by CC (hexane/ethyl acetate 8:2); yield: 87 % (127 mg). ^{1}H NMR (CD_3COCD_3): 8.76 (s, 1H), 8.36 (s, 1H), 7.98 (dd, *J* = 1.2 Hz and *J* = 4.4 Hz, 2H), 7.49 (d, *J* = 1.5 Hz, 1H), 7.43-7.37 (m, 4H), 7.15-7.13 (m, 1H), 6.87 (d, *J* = 8.2 Hz, 1H), 2.25 (s, 3H); ^{13}C NMR (CD_3COCD_3): 189.00, 159.30, 157.15, 145.25, 141.30, 134.75, 131.60, 130.70, 128.85, 128.25, 126.70, 122.25, 121.30, 117.40, 116.90, 17.20.

(2-Bromo-1,3-thiazol-5-yl)(3-methoxyphenyl)methanol (**22c**). The title compound was prepared by reaction of 2-bromo-1,3-thiazole-5-carbaldehyde (500 mg, 2.60 mmol) and 3-methoxyphenylmagnesium bromide (5.72 mmol) according to method F. The product was purified by CC (hexane/ethyl acetate 8:2); yield: 43 % (335 mg). ^{1}H NMR (CD_3COCD_3): 7.40 (s, 1H), 7.28 (t, *J* = 7.9 Hz, 1H), 7.08 (s, 1H), 7.03 (d, *J* = 7.9 Hz, 1H), 6.87-6.85 (m, 1H), 6.10 (d, *J* = 4.4 Hz, 1H), 5.55 (d, *J* = 4.4 Hz, 1H), 3.78 (s, 3H); ^{13}C NMR (CD_3COCD_3): 161.80, 151.05, 146.80, 140.90, 136.60, 131.40, 120.00, 115.05, 113.35, 71.20, 56.50.

(2-Bromo-1,3-thiazol-5-yl)(3-methoxyphenyl)methanone (**22b**). The title compound was prepared by reaction of (2-bromo-1,3-thiazol-5-yl)(3-methoxyphenyl)methanol (**22c**) (335 mg, 1.12 mmol) and 2-iodoxybenzoic acid (632 mg, 2.24 mmol) according to method G. The product was purified by CC (hexane/ethyl acetate 9:1); yield: 74 % (247 mg). ^{1}H NMR (CD_3COCD_3): 8.17 (s, 1H), 7.51-7.50 (m, 2H), 7.41 (s, 1H), 7.28-7.26 (m, 1H), 3.90 (s, 3H); ^{13}C NMR (CD_3COCD_3): 187.35, 161.90, 150.15, 145.10, 144.80, 140.10, 131.90, 123.25, 121.30, 115.35, 56.90.

[2-(4-Methoxy-3-methylphenyl)-1,3-thiazol-5-yl](3-methoxyphenyl)methanone (**22a**). The title compound was prepared by reaction of (2-bromo-1,3-thiazol-5-yl)(3-methoxyphenyl)methanone (**22b**) (247 mg, 0.83 mmol), 4-methoxy-3-methylbenzeneboronic acid (166 mg, 1.00 mmol), caesium carbonate (1.08 g, 3.32 mmol) and tetrakis(triphenylphosphine) palladium (9 mg, 8 μmol) according to method B. The product was purified by CC (hexane/ethyl acetate 9:1); yield: 23 % (65 mg). ^{1}H NMR ($CDCl_3$): 8.24 (s, 1H), 7.86 (dd, *J* = 2.2 Hz and *J* = 8.2 Hz, 1H), 7.84-7.83 (m, 1H), 7.48 (dt, *J* = 1.2 Hz and *J* = 7.6 Hz, 1H), 7.43 (t, *J* = 7.9 Hz, 1H), 7.41-7.40 (m, 1H), 7.16 (ddd, *J* = 0.9 Hz and *J* = 2.5 Hz and *J* = 8.2 Hz, 1H), 6.89 (d, *J* = 8.5 Hz, 1H), 3.91 (s, 3H), 3.88 (s, 3H), 2.29 (s, 3H); ^{13}C NMR ($CDCl_3$): 187.05, 174.90, 160.65, 159.80, 149.95, 137.75, 129.65, 129.35, 127.70, 126.40, 125.25, 121.55, 119.20, 113.40, 110.10, 55.55, 55.50, 16.20.

[2-(4-Hydroxy-3-methylphenyl)-1,3-thiazol-5-yl](3-hydroxyphenyl)methanone (**22**). The title compound was prepared by reaction of [2-(4-methoxy-3-methylphenyl)-1,3-thiazol-5-yl](3-methoxyphenyl)methanone (**22a**) (65 mg, 0.19 mmol) and pyridinium hydrochloride (1.63 g, 14.06 mmol) according to method E. The product was purified by CC (hexane/ethyl acetate 8:2) followed by preparative TLC (hexane/ethyl acetate 6:4); yield: 46 % (27 mg). ^{1}H NMR (CD_3COCD_3): 8.26 (s, 1H), 7.87-7.86 (m, 1H), 7.78 (dd, *J* = 2.2 Hz and *J* = 8.5 Hz, 1H), 7.43-7.40 (m, 2H), 7.38-7.37 (m, 1H), 7.16-7.14 (m, 1H), 6.97 (d, *J* = 8.2 Hz, 1H), 2.29 (s, 3H); ^{13}C NMR (CD_3COCD_3): 188.25, 176.30, 161.20, 159.65, 151.65, 141.25, 131.75, 131.60, 128.20, 127.40, 126.35, 121.90, 121.65, 117.20, 17.15.

(4-Bromo-1,3-thiazol-2-yl)(3-methoxyphenyl)methanol (**23c**). The title compound was prepared by reaction of 4-bromo-1,3-thiazole-2-carbaldehyde (500 mg, 2.60 mmol) and 3-methoxyphenylmagnesium bromide (5.72 mmol) according to method F. The product was purified by CC (hexane/ethyl acetate 9:1); yield: 74 % (580 mg). ^{1}H NMR (CD_3COCD_3): 7.53 (s, 1H), 7.27 (t, *J* = 7.9 Hz, 1H), 7.10-7.09 (m, 1H), 7.09-7.06 (m, 1H), 6.86 (ddd, *J* = 0.9 Hz and *J* = 2.5 Hz and *J* = 8.2 Hz, 1H), 6.03 (s, 1H), 5.91 (s, 1H), 3.78 (s, 3H); ^{13}C NMR (CD_3COCD_3): 178.85, 161.70, 145.40, 131.25, 125.60, 120.55, 119.45, 115.00, 114.05, 74.90, 56.50.

(4-Bromo-1,3-thiazol-2-yl)(3-methoxyphenyl)methanone (**23b**). The title compound was prepared by reaction of (4-bromo-1,3-thiazol-2-yl)(3-methoxyphenyl)methanol (**23c**) (580 mg, 1.93 mmol) and 2-iodoxybenzoic acid (1.09 g, 3.86 mmol) according to method G. The product was purified by CC (hexane/ethyl acetate 9:1); yield: 52 % (301 mg). ^{1}H NMR (CD_3COCD_3): 8.13 (s, 1H), 8.09-8.07 (m, 1H), 7.98-7.97 (m, 1H), 7.49 (t, *J* = 7.9 Hz, 1H), 7.27 (ddd, *J* = 0.9 Hz and *J* = 2.5 Hz and *J* = 8.2 Hz, 1H), 3.89 (s, 3H); ^{13}C NMR (CD_3COCD_3): 183.70, 169.50, 161.55, 137.50, 131.45, 128.10, 125.30, 121.80, 117.45, 56.80.

[4-(4-Methoxy-3-methylphenyl)-1,3-thiazol-2-yl](3-methoxyphenyl)methanone (**23a**). The title compound was prepared by reaction of (4-bromo-1,3-thiazol-2-yl)(3-methoxyphenyl)methanone (**23b**) (200 mg, 0.67 mmol), 4-methoxy-3-methylbenzeneboronic acid (133 mg, 0.80 mmol), caesium carbonate (873 mg, 2.68 mmol) and tetrakis(triphenylphosphine) palladium (8 mg, 7 µmol) according to method B. The product was purified by CC (hexane/ethyl acetate 9:1); yield: 66 % (150 mg). ^{1}H NMR (CD_3COCD_3): 8.23 (q, *J* = 1.5 Hz, 1H), 8.18 (ddd, *J* = 0.9 Hz and *J* = 1.5 Hz and *J* = 7.6 Hz, 1H), 8.12 (s, 1H), 7.83 (dd, *J* = 2.2 Hz and *J* = 8.2 Hz, 1H), 7.81-7.80 (m, 1H), 7.49 (t, *J* = 7.9 Hz, 1H), 7.26 (ddd, *J* = 0.9 Hz and *J* = 2.5 Hz and *J* = 8.2 Hz, 1H), 6.97 (d, *J* = 8.2 Hz; 1H), 3.92 (s, 3H), 3.86 (s, 3H), 2.23 (s, 3H); ^{13}C NMR (CD_3COCD_3): 184.50, 168.85, 161.50, 160.20, 159.40, 138.25, 131.30, 130.45, 128.40, 128.05, 127.15, 125.30, 121.85, 120.70, 117.40, 112.10, 56.85, 56.80, 17.50.

[4-(4-Hydroxy-3-methylphenyl)-1,3-thiazol-2-yl](3-hydroxyphenyl)methanone (**23**). The title compound was prepared by reaction of [4-(4-methoxy-3-methylphenyl)-1,3-thiazol-2-yl](3-methoxyphenyl)methanone (**23a**) (80 mg, 0.24 mmol) and boron tribromide (1.44 mmol) according to method D. The product was purified by CC (hexane/ethyl acetate 8:2); yield: 38 % (28 mg). ^{1}H NMR (CD_3COCD_3): 8.17 (s, 1H), 8.13 (ddd, *J* = 0.9 Hz and *J* = 1.5 Hz and *J* = 7.6 Hz, 1H), 8.09-8.08 (m, 1H), 7.84-7.83 (m, 1H), 7.78-7.75 (m, 1H), 7.45 (t, *J* = 8.2 Hz, 1H), 7.20 (ddd, *J* = 0.9 Hz and *J* = 2.5 Hz and *J* = 8.2 Hz, 1H), 6.93 (d, *J* = 8.2 Hz; 1H), 2.28 (s, 3H); ^{13}C

NMR (CD_3COCD_3): 185.00, 168.85, 159.80, 159.15, 157.95, 138.45, 131.45, 130.95, 127.55, 127.25, 126.55, 124.40, 122.60, 120.20, 119.20, 116.75, 17.30.

(2-Bromo-1,3-thiazol-4-yl)(3-methoxyphenyl)methanol (**24c**). The title compound was prepared by reaction of 2-bromo-1,3-thiazole-4-carbaldehyde (450 mg, 2.34 mmol) and 3-methoxyphenylmagnesium bromide (5.15 mmol) according to method F. The product was purified by CC (hexane/ethyl acetate 8:2); yield: 30 % (210 mg). ^{1}H NMR (CD_3COCD_3): 7.48 (d, J = 0.9 Hz, 1H), 7.23 (t, J = 7.9 Hz, 1H), 7.05-7.04 (m, 1H), 7.02-7.00 (m, 1H), 6.82 (ddd, J = 0.9 Hz and J = 2.5 Hz and J = 8.2 Hz, 1H), 5.87 (d, J = 4.4 Hz, 1H), 5.06 (d, J = 4.4 Hz, 1H), 3.77 (s, 3H); ^{13}C NMR (CD_3COCD_3): 171.90, 162.55, 161.65, 146.55, 131.00, 120.90, 120.40, 114.50, 114.35, 74.15, 56.45.

[2-(4-Methoxy-3-methylphenyl)-1,3-thiazol-4-yl](3-methoxyphenyl)methanol (**24b**). The title compound was prepared by reaction of (2-bromo-1,3-thiazol-4-yl)(3-methoxyphenyl)methanol (**24c**) (210 mg, 0.70 mmol), 4-methoxy-3-methylbenzeneboronic acid (139 mg, 0.84 mmol), caesium carbonate (684 mg, 2.10 mmol) and tetrakis(triphenylphosphine) palladium (8 mg, 7 μmol) according to method B but refluxed for 4h. The product was purified by CC (hexane/ethyl acetate 8:2); yield: 67 % (160 mg). ^{1}H NMR (CD_3COCD_3): 7.76-7.73 (m, 2H), 7.28 (d, J = 0.9 Hz, 1H), 7.23 (t, J = 7.9 Hz, 1H), 7.15-7.14 (m, 1H), 7.10-7.09 (m, 1H), 6.96 (d, J = 8.5 Hz, 1H), 6.81 (ddd, J = 0.9 Hz and J = 2.5 Hz and J = 8.2 Hz, 1H), 5.95 (d, J = 4.7 Hz, 1H), 4.97 (d, J = 4.7 Hz, 1H), 3.86 (s, 3H), 3.77 (s, 3H), 2.22 (s, 3H); ^{13}C NMR (CD_3COCD_3): 169.45, 162.95, 161.55, 161.30, 147.25, 130.85, 130.20, 128.65, 128.05, 127.25, 120.90, 114.70, 114.35, 114.30, 112.05, 74.40, 56.90, 56.40, 17.25.

[2-(4-Methoxy-3-methylphenyl)-1,3-thiazol-4-yl](3-methoxyphenyl)methanone (**24a**). The title compound was prepared by reaction of [2-(4-methoxy-3-methylphenyl)-1,3-thiazol-4-yl](3-methoxyphenyl)methanol (**24b**) (160 mg, 0.47 mmol) and 2-iodoxybenzoic acid (265 mg, 0.94 mmol) according to method G. The product was purified by CC (hexane/ethyl acetate 95:5); yield: 68 % (109 mg). ^{1}H NMR ($CDCl_3$): 8.11 (s, 1H), 7.90-7.88 (m, 1H), 7.84-7.83 (m, 1H), 7.83-7.79 (m, 2H), 7.41 (t, J = 7.9 Hz, 1H), 7.15 (ddd, J = 0.9 Hz and J = 2.5 Hz and J = 8.2 Hz, 1H), 6.87 (d, J = 8.5 Hz, 1H), 3.89 (s, 3H), 3.88 (s, 3H), 2.27 (s, 3H); ^{13}C NMR ($CDCl_3$): 186.75, 168.35, 159.85, 159.40, 154.90, 138.60, 129.15, 129.05, 127.40, 127.10, 125.85, 125.35, 123.30, 119.50, 114.70, 109.95, 55.45, 55.40, 16.15.

[2-(4-Hydroxy-3-methylphenyl)-1,3-thiazol-4-yl](3-hydroxyphenyl)methanone (**24**). The title compound was prepared by reaction of [2-(4-methoxy-3-methylphenyl)-1,3-thiazol-4-yl](3-methoxyphenyl)methanone (**24a**) (109 mg, 0.32 mmol) and pyridinium hydrochloride (2.75 g,

23.68 mmol) according to method E. The product was purified by CC (hexane/ethyl acetate 7:3); yield: 44 % (44 mg). ^{1}H NMR (CD_3COCD_3): 8.76 (s, 1H), 8.73 (s, 1H), 8.29 (s, 1H), 7.81-7.78 (m, 3H), 7.74 (dd, *J* = 2.2 Hz and *J* = 8.5 Hz, 1H), 7.39 (t, *J* = 7.9 Hz, 1H), 7.13 (ddd, *J* = 0.9 Hz and *J* = 2.5 Hz and *J* = 8.2 Hz, 1H), 6.96 (d, *J* = 8.5 Hz, 1H), 2.28 (s, 3H); ^{13}C NMR (CD_3COCD_3): 188.05, 171.95, 170.10, 159.85, 159.10, 156.85, 140.75, 131.20, 128.70, 127.65, 127.20, 126.80, 123.75, 121.80, 118.80, 117.05, 17.10.

Biological Methods

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

17β-HSD1 and 17β-HSD2 were obtained from human placenta according to previously described procedures.[38] Fresh human placenta was homogenized and cytosolic fraction and microsomes were separated by 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 17β-HSD1

Inhibitory activities were evaluated by an established method with minor modifications.[38] Briefly, the enzyme preparation was incubated with NADH [500 µM] in the presence of potential inhibitors at 37 °C in a phosphate buffer (50 mM) supplemented with 20 % of glycerol and EDTA (1 mM)**.** Inhibitor stock solutions were prepared in DMSO. The final concentration of DMSO was adjusted to 1 % in all samples. The enzymatic reaction was started by addition of a mixture of unlabelled- and [2, 4, 6, 7-^{3}H]-E1 (final concentration: 500 nM, 0.15 µCi). After 10 min, the incubation was stopped with $HgCl_2$ and the mixture was extracted with diethylether. After evaporation, the steroids were dissolved in acetonitrile. E1 and E2 were separated using acetonitrile/water (45:55) as mobile phase in a C18 reverse phase chromatography column (Nucleodur C18 Gravity, 3 µm, Macherey-Nagel, Düren) connected to a HPLC-system (Agilent 1100 Series, Agilent Technologies, Waldbronn). Detection and quantification of the steroids were performed using a radioflow detector (Berthold Technologies, Bad Wildbad). The conversion rate was calculated after analysis of the resulting chromatograms according to the following equation: $\%conversion = \frac{\%E2}{\%E2 + \%E1} \times 100$. Each value was calculated from at least three independent experiments.

Inhibition of 17β-HSD2

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

The conversion rate was calculated after analysis of the resulting chromatograms according to the following equation: $\%conversion = \frac{\%E1}{\%E1 + \%E2} \times 100$.

ER affinity

The binding affinity of selected compounds to the ERα and ERβ was determined according to Zimmermann et al.[54] Briefly, 0.25 pmol of ERα or ERβ, respectively, were incubated with [2, 4, 6, 7-^{3}H]-E2 (10 nM) and test compound for 1 h at room temperature. The potential inhibitors were dissolved in DMSO (5 % final concentration). Evaluation of non-specific-binding was performed with diethylstilbestrol (10 µM). After incubation, ligand-receptor complexes were selectively bound to hydroxyapatite (5 g/ 60 mL TE-buffer). The complex formed was separated, washed and resuspended in ethanol. For radiodetection, scintillator cocktail (Quickszint 212, Zinsser Analytic, Frankfurt) was added and samples were measured in a liquid scintillation counter (Rack Beta Primo 1209, Wallac, Turku). For determination of the relative binding affinity (RBA), inhibitor and E2 concentrations required to displace 50 % of the receptor bound labelled E2 were determined. RBA values were calculated according to the following equation: $RBA[\%] = \frac{IC_{50}(E2)}{IC_{50}(compound)} \times 100$. The RBA value for E2 was arbitrarily set at 100 %.

Inhibition of 17β-HSD1 in T47D cells

A stock culture of T47D cells was grown in RPMI 1640 medium supplemented with 10 % FCS, L-glutamine (2 mM), penicillin (100 IU/mL), streptomycin (100 µg/mL), insulin-zinc-salt (10 µg/mL) and sodium pyruvate (1 mM) at 37 °C under 5 % CO_2 humidified atmosphere.

The cells were seeded into a 24-well plate at 1x10^6 cells/well in DMEM medium with FCS, L-glutamine and the antibiotics added in the same concentrations as mentioned above. After 24 h the medium was changed for fresh serum free DMEM and a solution of test compound in DMSO was added. Final concentration of DMSO was adjusted to 1 % in all samples. After a pre-incubation of 30 min at 37 °C with 5 % CO_2, the incubation was started by addition of a mixture of unlabelled- and [2, 4, 6, 7-^{3}H]- E1 (final concentration: 50 nM, 0.15 µCi). After 0.5 h incubation, the enzymatic reaction was stopped by removing of the supernatant medium. The

steroids were extracted with diethylether. Further treatment of the samples was carried out as mentioned for the 17β-HSD1 assay.

Metabolic stability

Human liver microsomes (final protein concentration 0.5 mg/mL), 0.1 M phosphate buffer pH 7.4 and test compound (final substrate concentration = 3 µM; final DMSO concentration = 0.25 %) were preincubated at 37 °C prior to the addition of NADPH (final concentration = 1 mM) to initiate the reaction. Dextromethorphan and verapamil were used as references. All incubations were performed singularly for each test compound. Each compound was incubated for 0, 5, 15, 30 and 45 min (control: 45 min). The reactions were stopped by the addition of 50 µL methanol containing internal standard at the appropriate time points. The incubation plates were centrifuged at 2000 rpm for 20 min at 4 °C to precipitate the protein. The sample supernatants were combined in cassettes of up to 4 compounds and analyzed using LC-MS/MS.

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3.3 Bicyclic substituted Hydroxyphenylmethanone Type Inhibitors of 17β-Hydroxysteroid Dehydrogenase Type 1 (17β-HSD1): The Role of the Bicyclic Moiety

Alexander Oster, Tobias Klein, Claudia Henn, Ruth Werth, Sandrine Marchais-Oberwinkler, Martin Frotscher, Rolf W. Hartmann

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Publikation III

Abstract

An upcoming target for the treatment of estrogen-dependent diseases, such as breast cancer and endometriosis, is the enzyme responsible for the last step in the biosynthesis of estradiol (E2): 17β-hydroxysteroid dehydrogenase type 1 (17β-HSD1). It catalyzes the reduction of the weakly active estrone (E1) into E2, which is the most potent estrogen in humans. Inhibition of 17β-HSD1 lowers intracellular E2 concentrations and thus presents a new therapy option for estrogen-dependent pathologies. Recently, we have reported on a new class of highly active and selective 17β-HSD1 inhibitors: bicyclic substituted hydroxyphenylmethanones. Here, further structural variations on the bicyclic moiety were performed, especially focusing on the exchange of its OH-function. Twenty-nine novel inhibitors were synthesized and evaluated for 17β-HSD1 inhibition in a cell-free and cellular assay, for selectivity toward 17β-HSD2 and estrogen receptors (ER) α and β as well as for metabolic stability. Compound **23** turned out to be the best compound of this study with excellent IC_{50}-values of 12 nM in the cell-free assay and 78 nM in the cellular assay, a high selectivity, and a reasonable stability. A molecular docking study provided insight into the protein-ligand interactions of **23**.

Introduction

17β-Hydroxysteroid dehydrogenases (17β-HSDs) are responsible for the oxido-reduction reactions of steroid hormones at the 17 position, especially for estrogens and androgens, which use NAD(P)H or $NAD(P)^+$ as cofactors. Although this is a reversible reaction, under in vivo

conditions the catalysis is unidirectional due to the fact that $NADP^+$ is abundant in its reduced form, whereas NAD^+ is mainly present in its oxidized form.[1] 17β-HSDs catalyze the conversion of hormones with high receptor affinity to the corresponding low affinity forms and *vice versa*. 17β-HSDs are expressed in the gonads and show characteristic expression patterns in peripheral tissues.[2] Therefore they play a pivotal role in the local regulation of hormone levels.[2] In addition to their individual tissue and subcellular distributions, they also differ in their substrate specificities.[3, 4]

17β-HSD1 is the most extensively characterized 17β-HSD subtype. It catalyzes the last step in the biosynthesis of the highly active estrogen 17β-estradiol (E2): the reduction of the weakly active estrone (E1). (Figure 1)

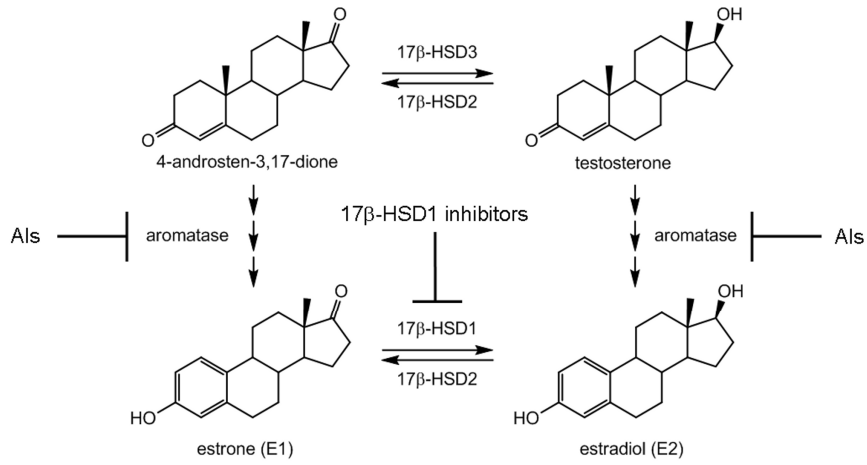


***Figure 1*: Intervention of AIs and 17β-HSD1 inhibitors in the estrogen synthesis**

It is well known that E2, the natural ligand of the estrogen receptors α and β (ERα and ERβ), has a high impact on the development and progression of estrogen dependent diseases such as breast cancer, endometriosis, and endometrial hyperplasia.[5-7] Reduction of estrogen levels by interference with E2-biosynthesis has been used as a treatment of ER-positive postmenopausal breast cancer for almost thirty years. Up to now, aromatase inhibitors (AIs) are firstline therapeutics for these patients.[8, 9] (Figure 1) In the past few years, AIs have been intensively investigated.[10-15] However, the limitations of AIs such as resistance and further disease progression come more and more to the forefront.[16, 17] Furthermore, inhibition of E2 synthesis by AIs does not affect the last but only the penultimate step. It results in a strong reduction of estrogen levels in the whole body leading to unwanted side effects. Therefore, intracellular regulation of estrogen levels in the diseased tissues by 17β-HSD1 is a promising new therapeutic approach for the treatment of estrogen dependent diseases. This concept has already been demonstrated as successful with the use of 5α-reductase inhibitors in the treatment of androgen dependent diseases such as benign prostatic hyperplasia and alopecia.[18-21] Proof of

concept for the treatment of breast cancer has already been performed in different mouse-models.[22-24] However, no 17β-HSD1 inhibitor has reached clinical trials so far. Furthermore, inhibition of 17β-HSD1 could have an additional antitumor effect, because it is also involved in the metabolism of retinoic acid,[25, 26] a natural compound known to have tumor inhibiting activity.[27] As the therapeutic efficacy of 17β-HSD1 inhibitors should not be counteracted, selectivity towards 17β-hydroxysteroid dehydrogenase type 2 (17β-HSD2) is required. This enzyme catalyzes the reverse reaction: oxidation of E2 into E1. Additionally, for reducing the risk of intrinsic estrogenic effects, affinity of the inhibitors to the ERs should be avoided. In recent years, the structural architecture of 17β-HSD1 has been actively investigated and numerous crystallographic data have been determined. This information is of great value for the identification of potential inhibitors that employ molecular modeling methods, as well as for structure-based design. Drug design efforts will further be facilitated by recently published first insights in 17β-HSD1 enzyme kinetics and ligand binding by dynamic motion investigations.[28]
The enzyme consists of 327 amino acids and exists as a homodimer with a subunit mass of 34.9 kDa.[29] 17β-HSD1 belongs to the superfamiliy of short-chain dehydrogenases/reductases (SDRs) and is characterized by the presence of the Rossmann fold, a protein structural motif that is relevant for cofactor binding.[29] Closer analysis of the active site reveals that the substrate binding pocket can be divided into three main regions. The C-terminal region contains His221 and Glu282, which are responsible for hydrogen bonding with the O3 of the steroidal A-ring. A mainly hydrophobic region, often described as hydrophobic tunnel, is responsible for binding the central apolar core of the steroid. The third area is the catalytic region containing the amino acids Ser142 and Tyr155, which form a hydrogen bond network with the steroidal D-ring O17.
Several compounds have already been published as 17β-HSD1 inhibitors[26, 31-34] most of them with steroidal structures. With regards to non-steroidal inhibitors, there are three compound classes described showing promising inhibitory profiles, namely thiophenepyrimidinones,[35, 36] hydroxyphenylnaphthols[37-40] and bis(hydroxyphenyl)heterocycles.[41-45] Recently, we reported on bicyclic substituted hydroxyphenylmethanones as a new class of 17β-HSD1 inhibitors[46] (Figure 2 A).

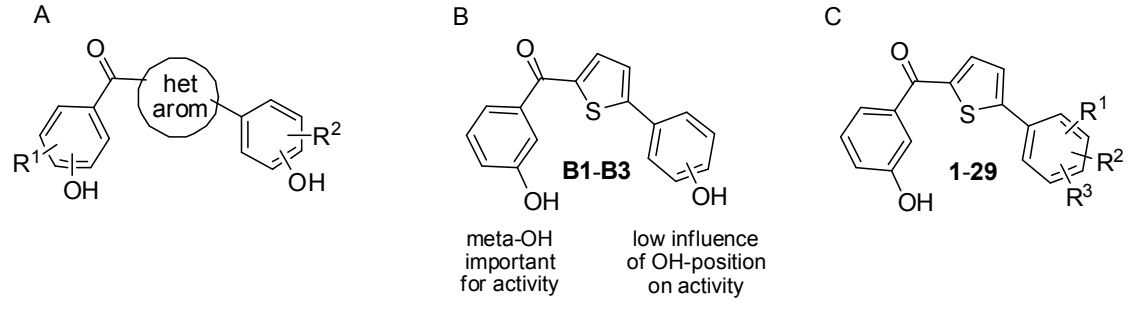
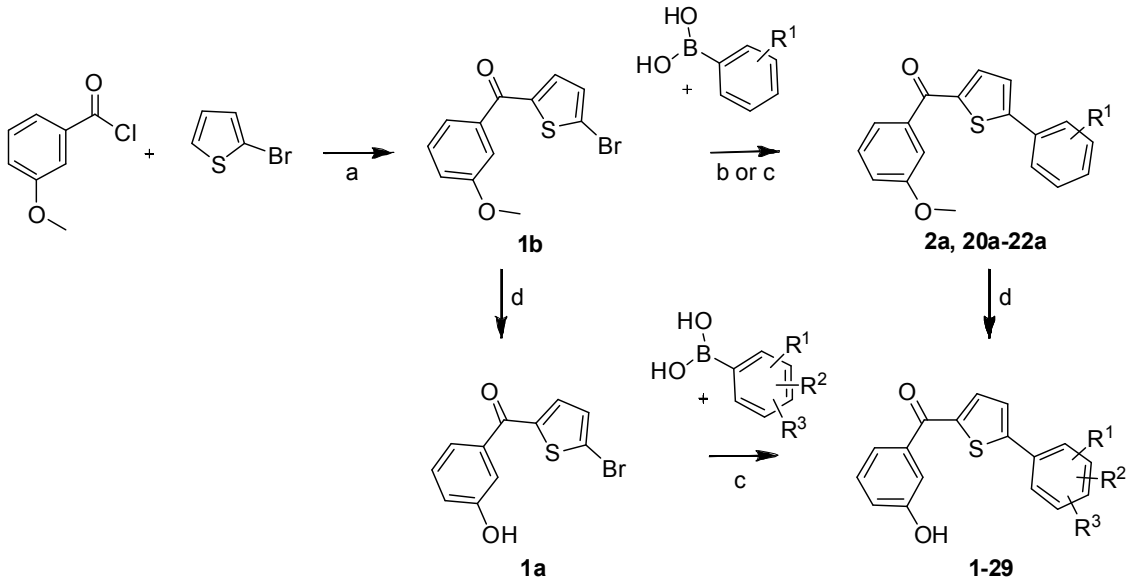


***Figure 2:* Bicyclic substituted hydroxyphenylmethanones as inhibitors of 17β-HSD1**

Binding mode investigations of three potent compounds of this class (**B1**-**B3)** revealed that they interact with an additional mainly apolar subpocket, consisting of amino acids Leu95, Leu96, Asn152, Tyr155 and Phe192. The pocket is located below the catalytic region and was previously described to have an impact on ligand binding.[47, 48] The hydroxyphenylmethanone part is considered to serve two purposes: the ketone mimics the D-ring keto function of the natural substrate, while the hydroxyphenyl moiety occupies the subpocket and forms an additional H-bond with Asn152. The introduction of further substituents (larger than fluorine) on the benzoyl ring, as well as the variation of the OH-position on this ring, led to a decrease of inhibitory activity. Conversely, modifications of the OH-position on the phenyl ring do not influence the activity significantly (Figure 2 B). While this OH-group was originally expected to interact with His221 and Glu282, its relevance for ligand-binding remained unclear.
In the following, we will report on the modification of the phenyl ring in order to search for possible substitutes for the OH-function and to shed light on the influence of other substituents on 17β-HSD1 inhibition. In view of the fact that phenols are known for their susceptibility to phase II metabolism,[49] structural changes are also desirable. Synthesis, biological evaluation and structure-activity relationships of new highly potent and selective 17β-HSD1 inhibitors will be described. Furthermore, the binding mode of the most potent compound was investigated by molecular docking studies to explain the SAR obtained.

Chemistry

The synthesis of compounds **1**-**29** is depicted in Scheme 1. The methoxylated intermediate **1b** was synthesized via Friedel-Crafts-acylation of 2-bromothiophene with 3-methoxybenzoyl chloride using aluminium chloride. The methoxy function was cleaved according to method B (BBr_3, CH_2Cl_2, -78 °C to rt, 18 h)[50] to yield compound **1a**, quantitatively. Starting from these brominated key intermediates **1a** and **1b** and the appropriate commercially available boronic acids, Suzuki cross coupling reactions[51] were carried out using two different methods. Compound **2a** was prepared in a one-pot synthesis by means of microwave assisted Suzuki reaction (Cs_2CO_3, DME/EtOH/water (1:1:1), $Pd(PPh_3)_4$, MW (150 W, 150 °C, 15 bar 15 min)).[42] All other compounds were prepared following method A. Using DME/water (1:1) as solvent, caesium carbonate and $Pd(PPh_3)_4$ as catalyst, the reaction mixtures were stirred under reflux either for 2 h (method A1) for **1**, **3**-**19** and **23**-**28** or for 4 h (method A2) for intermediates **20a**-**22a** and **29**. Demethylation of **2a**, sulfonamide derivatives **21** and **22** and nitrile **20** was performed using method B (BBr_3, CH_2Cl_2, -78 °C to rt, 18 h).



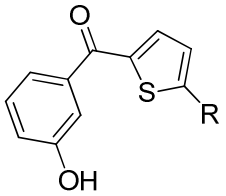
***Scheme 1*: Synthesis of compounds 1-29.**

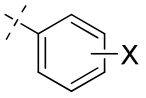
Reagents and conditions: a) $AlCl_3$, anhydrous CH_2Cl_2, 0 °C, 0.5 h followed by rt, 1 h for compound **1b**; b) Cs_2CO_3, $Pd(PPh_3)_4$, DME/EtOH/water (1:1:1), microwave conditions (150 W, 15 bar, 150 °C, 15 min) for compound **2a**; c): Cs_2CO_3, $Pd(PPh_3)_4$, DME/water (1:1) reflux, 4 h for compounds **20a**-**22a** and **29** (Method A2) and 2 h for compounds **1**, **3**-**19** and **23**-**28** (Method A1); d) Method B: BBr_3, CH_2Cl_2, -78 °C, 18 h for compounds **1a**, **2** and **20**-**22**.

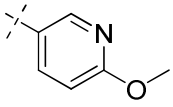
Biological results

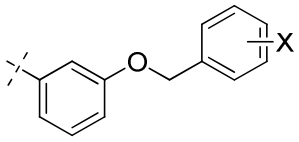
Activity: Inhibition of human 17β-HSD1

Human placental enzyme was used as enzyme source and partially purified following a described procedure.[52] Tritiated E1 was incubated with 17β-HSD1, cofactor and inhibitor. The separation of substrate and product was performed by HPLC. The inhibition values of compounds **1**-**29** are shown in Table 1.









B1-B3, **1-4**, **6-11**, **20-23** **5** **12-19** **24-29**

cmpd	X	IC_{50} [nM][a]		selectivity factor[d]
		17β-HSD1[b]	17β-HSD2[c]	
B1	2-hydroxy	95	18	0.2
B2	3-hydroxy	22	109	5
B3	4-hydroxy	33	478	14
1	H	155	128	0.8
2	2-methoxy	86	196	2
3	3-methoxy	95	342	4
4	4-methoxy	220	507	2
5		510	1036	2
6	3,4-dimethoxy	44 %[e]	44 %[e]	
7	3,4,5-trimethoxy	54 %[e]	26 %[e]	
8	2,3,4-trimethoxy	515	915	2
9	2-ethoxy	94	195	2
10	3-ethoxy	78	502	6
11	4-ethoxy	498	586	1
12	H	62	797	13
13	2-methoxy	35	1065	30
14	3-methoxy	47	768	16
15	4-methoxy	60	702	12
16	3,5-dimethoxy	31	789	26
17	2-chloro	54	1197	22
18	3-chloro	40	1034	26
19	4-chloro	36	1003	28
20	3-nitrile	225	354	2
21	3-methanesulfonamide	150	563	4
22	*N*-methylmethanesulfonamide	125	559	4
23	3-(4-methylbenzenesulfonamide)	12	169	14
24	2-naphthalene	300	253	0.8
25	5-(2,3-dihydrobenzofurane)	55	319	6
26	5-(1,3-benzodioxol)	66	299	5
27	5-indole	151	173	1
28	6-indole	192	283	2
29	6-indazole	107	410	4

***Table 1:* Inhibition of human 17β-HSD1 and 17β-HSD2 by compounds 1-29**

[a] Mean value of three determinations, standard deviation less then 26 %
[b] Human placental, cytosolic fraction, substrate E1, 500 nM, cofactor NADH, 500µM
[c] Human placental, microsomal fraction, substrate E2, 500 nM, cofactor NAD^+, 1500µM
[d] IC_{50} (17β-HSD2) / IC_{50} (17β-HSD1)
[e] inhibition at 1 µM (inhibitor concentration)

Compound **1**, lacking the OH-function on the phenyl ring, showed an inhibitory activity, which is not considerably weaker than the activities of the hydroxylated compounds **B1**-**B3**. This fact confirms the hypothesis that this OH-function is not necessary for high 17β-HSD1 inhibition and prompted us to synthesize the corresponding methoxy-derivatives **2**-**4**. As compound **2** (*ortho*-methoxy) and **3** (*meta*-methoxy) revealed remarkable IC_{50}-values around 90 nM, it became apparent that a hydrogen-bond donor is not necessarily needed in this region. The correspondent di- and trimethoxy-derivatives **6**-**8** showed a decrease in inhibitory potency. Taking in account that the monomethoxy-functions (**2**-**4**) led to high potency, the alkyl moiety was enlarged (**9**-**11**). The *ortho*- and *meta*-ethoxy-compounds **9** and **10** exhibited similar 17β-HSD1 inhibition as the corresponding methoxy-derivatives **2** and **3** indicating that there is some space for further substituents. However, an enlargement of substituents in *para*-position turned out to be unfavorable since *para*-ethoxy **11** showed reduced activity (IC_{50} = 498 nM) compared to **4** (IC_{50} = 220 nM). For exploring the space around the *meta*-position of the phenyl ring, compound **12** was synthesized bearing a *meta*-benzyloxy-function instead of ethoxy (**10**). The strong inhibitory activity of **12** (IC_{50} = 62 nM) indicates the presence of additional space, which was further investigated by the introduction of substituents on each position of the benzyloxy-moiety. Interestingly, the methoxy (**13**-**15**), dimethoxy (**16**) and chlorine (**17**-**19**) showed similar 17β-HSD1 inhibition indicating the benefit of a benzyloxy moiety for enzyme inhibition.

Considering the fact that high inhibitory activity can be achieved without OH-group on the phenyl ring (as seen with **1**), other substituents were introduced in *meta*-position. While the introduction of a carbonitrile (**20,** IC_{50} = 225 nM) or methanesulfonamide-group (**21**, IC_{50} = 150 nM and **22**, IC_{50} = 125 nM) did not induce significant changes in enzyme inhibition compared to the unsubstituted compound **1** (IC_{50} = 155 nM), the methylbenzenesulfonamide **23** was clearly more potent and exhibited the highest 17β-HSD1 inhibitory activity (IC_{50} = 12 nM) in this study.

Up to this point of the study, more or less flexible substituents were introduced into the phenyl ring. In the next step this ring was exchanged by different condensed bicyclic moieties. Compared to compound **1** (IC_{50} = 155 nM), the naphthalene-substituted derivative **24** showed a slight drop of activity (IC_{50} = 300 nM). Introduction of a dihydrobenzofuran moiety (**25**), which represents a rigidification of the ethoxy-substituent of compound **11** (IC_{50} = 498 nM) led to increased inhibition values (IC_{50} = 55 nM). Obviously, rigidified systems are more appropriate for inhibitory activity in this position of the molecule indicating a sterical hindrance for larger substituents in *para*-position. This observation was confirmed by comparing compounds **6** and **26**: by replacing the two methoxy-functions (**6**) with an acetale (**26**), the weak inhibition (44 % at 1 μM) could be increased to an IC_{50}-value of 66 nM. Since oxygen-containing bicyclic moieties are appropriate for high inhibitory activity, compounds **27**-**29** were synthesized in

order to evaluate whether nitrogen was a suitable alternative. Although showing remarkable activities, indoles **27** and **28** as well as indazole **29** were less active than **25** and **26**.

Selectivity: Inhibition of human 17β-HSD2 and affinities for ERα and ERβ

17β-HSD2 acts as a biological counterpart of the type 1 enzyme by catalyzing the reverse reaction. Therefore inhibition of this enzyme must be avoided. In an assay similar to the 17β-HSD1 test, human placental microsomes were incubated with tritiated E2 in the presence of NAD^+ and inhibitor to evaluate inhibition of 17β-HSD2. Labeled product was quantified after HPLC separation. Inhibition values of all compounds are depicted in Table 1. In vitro selectivity toward 17β-HSD2 is expressed as selectivity factor (SF), describing the ratio of the concentrations required to inhibit the isoenzymes by 50 % (IC_{50} 17β-HSD2 / IC_{50} 17β−HSD1).

SFs of methoxy- and ethoxy-compounds **1**-**2**, **4**-**9** and **11** do not exceed 2. Only in the case of compounds bearing substituents in *meta*-position, higher SFs were obtained. Interestingly, enlargement of the ether resulted in a slight increase of selectivity comparing **3** (SF = 4) to **10** (SF = 6). Remarkably, the benzyloxy-derivative **12** showed a 13-fold higher inhibition of 17β-HSD1 compared to the type 2 enzyme indicating that the space within this area of the latter enzyme might be more restricted than in 17β-HSD1. Accordingly, introduction of substituents into the benzyloxy moiety might lead to further enhancement of selectivity. While this hypothesis was confirmed by the *meta*-methoxy- (**13**), dimethoxy- (**16**) and the chloro-derivatives (**17**-**19**), the monomethoxy-compounds (**14**-**15**) showed different results. Compounds **14** (*meta*-methoxy, SF = 16) and **15** (*para*-methoxy, SF = 12) revealed similar selectivity toward 17β-HSD2 as the non-substituted benzyloxy compound **12** (SF = 13). Although having similar sizes, the influence of chloro- and methoxy-substituents is different indicating that not only sterical parameters are responsible for the higher selectivity. Electronic effects might also be implicated.

Considering the sulfonamides **21**-**23**, it is striking that the introduction of a phenyl ring also increases selectivity (SF **21** and **22** = 4 whereas SF **23** = 14). These results attract more and more notice to a phenyl ring within this area of the protein with regard to his influence on activity and selectivity.

The condensed bicyclic derivatives **24**-**29** revealed no further enhancement of selectivity toward 17β-HSD2. While compound **25** showed the best SF = 6 within this rather rigid class of compounds, the naphthalene **24** inhibited both 17β-HSD subtypes with nearly the same potency.

As estrogens function via activation of ERs, the affinity of 17β-HSD1 inhibitors toward ERα and ERβ should be as low as possible in order to avoid stimulatory effects. Since E1 shows 10-fold lower affinity toward ERα[53] and 50-fold lower affinity toward ERβ[53] compared to E2

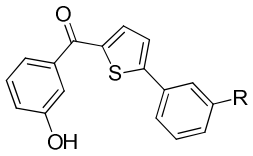
(relative binding affinity, RBA, of E2 = 100 %), inhibitors having a RBA below 0.1 % were considered to possess too little affinity to exert estrogenic effects. As E1 is the only active estrogen present during 17β-HSD1 inhibition, inhibitors with much lower receptor affinity than E1 are negligible for receptor activation. All compounds having a SF higher than 10 were evaluated in this competition assay. None of them showed higher RBA values than 0.1 % (data not shown) confirming our design concept focusing on non-steroidal inhibitors.

Further biological evaluations

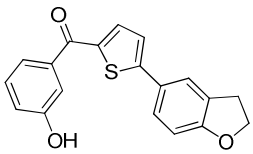
Using T47D cells expressing both 17β-HSD1 and 17β-HSD2, intracellular potency of selected compounds was evaluated (Table 2). Regarding the benzyloxy-substituted compounds **12-19**, only the monomethoxy-substituted benzyloxy-derivatives **13-15** showed moderate inhibition of E2 formation (IC_{50} = 526, 441 and 565 nM, respectively). However, the dimethoxy- (**16**) and chloro-substituents (**17-19**) have a negative impact on cellular activity, which might be explained by an insufficient cell penetration or an increased intracellular metabolism. Interestingly, the ethoxy derivative (**10**) without bearing an additional phenyl ring, exhibited a 3-4 fold higher intracellular inhibitory activity (IC_{50} = 154 nM) than compounds **13-15**.

Methylbenzenesulfonamide **23** turned out to be the strongest 17β-HSD1 inhibitor of this study both in the cell-free (IC_{50} = 12 nM) and in the cellular assay (IC_{50} = 78 nM). In contrast to the benzyloxy-compounds, high intracellular inhibitory activity was obtained despite the additional phenyl ring.

In order to find an appropriate species for demonstrating in vivo efficacy in an disease oriented animal model, compounds **1**, **13** and **23** were tested in a cell-free assay using the cytosolic fraction of mouse liver preparation and similar conditions to our standard cell-free assay. However, only neglectable inhibition of 17β-HSD1 or 17β-HSD2, could be detected (data not presented). Therefore, the search for an optimal species pertaining to in vivo proof of concept for this class of compounds is ongoing.



10, **12-19**, **23**



25

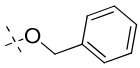
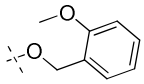
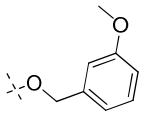
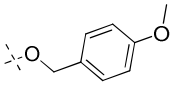
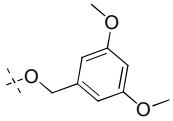
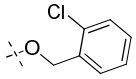
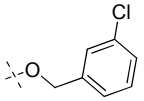
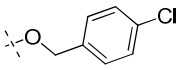
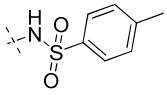
cmpd	R	IC_{50} [nM][a]	
		cell-free assay[b]	cellular assay[c]
10		78	154
12		62	53 %[d]
13		35	441
14		47	565
15		60	526
16		31	40 %[d]
17		54	34 %[d]
18		40	49 %[d]
19		36	47 %[d]
23		12	78
25		55	357

***Table 2:* Inhibition of 17β-HSD1 in cell-free and cellular assay by selected compounds**

[a] Mean values of three determinations, standard deviation less then 24 %
[b] Human placental, cytosolic fraction, substrate E1, 500 nM, cofactor NADH, 500µM
[c] T47D cells, substrate E1, 50 nM
[d] inhibition at 1 µM (inhibitor concentration)

Furthermore, metabolic stability of the most active compound **23** (IC_{50} = 12 nM) and the most selective compound **13** (SF = 30) of this study were evaluated using human liver microsomes (Table 3). The data of both compounds and the references dextromethorphan and verapamil are given in Table 3. **13** and **23** showed intrinsic clearances (CLint) of 81.2 and 109 µL/min/mg protein, respectively, and therefore they can be arranged in the high clearance category. Regarding the references, the often prescribed calcium channel blocker verapamil revealed a very similar CLint of 105 µL/min/mg protein defining a high but not too high liability to metabolic degradation.

cmpd	CLint[a] [µL/min/mg protein]	$t_{1/2}$ [min]
dextromethorphan	10.7	129
verapamil	105	13.2
13	81.2	17.1
23	109	12.8

Table 3: ***Metabolic stability of compound 14 and control compounds using human liver microsomes***
[a] Test concentration = 3 µM

Molecular Modeling

A docking study was performed in order to gain deeper insight into the molecular interactions between the most potent inhibitor **23** and 17β-HSD1 and to understand the SAR. In Figure 3, the binding mode of compound **23** is shown, indicating H-bond interactions between the OH-function and Asn152, as well as between the keto-oxygen and Tyr155. The second H-bond of the ketone with Ser142, which was observed for **B1**-**B3**, was not found for **23**. The methylbenzenesulfonamide substituent interacts with two amino acids, which are located in the C-terminal region. His221 forms a hydrogen bond with one sulfonamide-oxygen, while Phe259 might be involved in π-π-interactions with the benzene moiety.

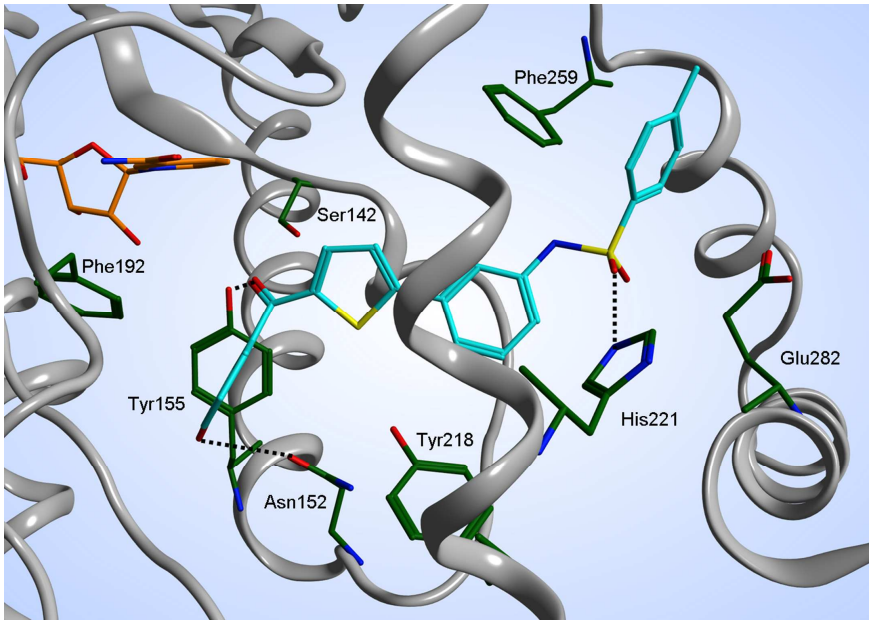


Figure 3: ***Hypothetical binding mode of compound 23 (cyan) obtained by docking into 17□-HSD1 (PDB-ID: 1FDT-B). Cofactor NADPH (orange), interacting residues (green) and cartoon rendered tertiary structure (grey) of the active site are shown. Hydrogen bonds are drawn in black dashed lines. Figure generated with MOE (Chemical Computing Group Inc., Montreal, Canada).***

Discussion and Conclusion

From a previous work in this class of compounds,[46] it was demonstrated that variations of the OH-position on the phenyl ring do not result in relevant differences regarding inhibitory activity of evaluated compounds **B1**-**B3**. In this paper, the biological data obtained confirm the hypothesis that the OH-function on the phenyl ring does not play an important role for 17β-HSD1 inhibition. Considering the original design concept, this hydroxy-group was expected to form hydrogen bonds with His221 and Glu282 in the C-terminal region of the steroid binding pocket. Obviously, the H-bond donor property is not necessary for strong inhibitory activity with regard to the methoxy-derivatives. Glu282, which is expected to accept a hydrogen bond from this OH-group, does not seem to be involved in inhibitor binding at all. In general, its relevance for ligand binding is discussed controversially. While site-directed mutagenesis studies indicated no significant interaction between Glu282 and the 3-OH group of the substrate,[54] H-bonding between both was found in several independent crystal structures.[55, 56] The H-bond acceptor property of the phenol might play a more important role since compound **1**, without hydroxy-function on the phenyl, showed a slightly reduced 17β-HSD1 inhibition. However, the potential H-bond donating His221 is already known as an important factor for substrate interaction.[57] Although this amino acid is described to be implicated in enzyme stabilisation[47] by interacting with Glu282, it appears to also be involved in the binding of our inhibitors.

Regarding the mono-methoxy- and ethoxy-derivatives (**2**-**5** and **9**-**11**, respectively), it becomes apparent that the *para*-position seems to be less favorable than *meta* and *ortho*. Moreover, di- and trimethoxy-derivatives (**6**-**8**) showed a strong decrease of activity as well, indicating that the space around this phenyl ring is sterically restricted. This finding is in accordance with our docking results. According to the orientation of the phenyl ring, the disubstitution of **6** forces the methoxy groups to adopt a conformation leading to sterical clashes with either Tyr218 or Phe259. Indeed, taking into account that the 1,3-benzodioxol compound **26** showed a much higher affinity than the corresponding dimethoxy compound **6**, it can be concluded that the binding pocket is very restricted. In general, compounds containing condensed rings, instead of the phenyl ring, showed a good inhibitory profile, especially the oxygen-containing **25** and **26**. Only naphthalene **24** revealed a slightly higher IC_{50}-value of 300 nM.

Concerning flexible substituents, 17β-HSD1 inhibitory activity as well as selectivity toward the type 2 enzyme revealed the *meta*-position to be the most favorable. Starting with compound 3, it is striking that an enlargement of the *meta*-substituents resulted step by step in an increased selectivity (SF of **3** < **10** < **12** < **13**, **16**-**19**). Interestingly, the IC_{50}-values for 17β-HSD1 inhibition of all benzyloxy-derivatives are in the same range and similar to the one of the ethoxy compound **10**. Therefore, although influencing the selectivity toward 17β-HSD2, the phenyl

ring of the benzyloxy substituent does not seem to specifically interact with 17β-HSD1. Further investigation is still warranted as to whether the phenyl ring is pointing out of the 17β-HSD1 active site.

Sulfonamide **23** turned out to be the strongest inhibitor of this study. Compared to its methanesulfonamide analog **21**, the introduction of the phenyl led to 12-fold higher 17β-HSD1 inhibition. The docking study revealed the benzene ring of **23** to be implicated in a π-π interaction with Phe259. Comparing **B2** (*meta*-OH) and **23**, both compounds interact with Asn152. The relevance of this amino acid for ligand binding was already confirmed by a cocrystal structure of a highly potent, steroidal 17β-HSD1 inhibitor.[47] **B2** and **23** showed similar IC_{50}-values although the H-bond interaction of **23** with Ser142 was not present in the observed binding mode. The π-π interactions found for compound **23** might be able to compensate the loss of the hydrogen bond between the ketone and Ser142.

In summary, we reported on further developments in the class of bicyclic substituted hydroxyphenylmethanones. Focusing on compounds bearing only one hydroxy-group, synthesis and evaluation of their biological properties were described. Investigations in structural changes concerning the phenyl ring demonstrate that potential inhibitors can abstain from one OH-function to exhibit 17β-HSD1 inhibition values in the low nanomolar range. This second OH-function can be replaced by even large substituents like, for instance, (substituted) benzyloxy side chains. The most promising compound of this study, methylbenzenesulfonamide **23**, shows strong inhibitory activity in both cellular and cell-free assays as well as no affinity toward estrogen receptors α and β. It might be a candidate for further lead optimization.

Experimental section

Chemical methods

Chemical names follow IUPAC nomenclature. Starting materials were purchased from Aldrich, Acros, Lancaster, Combi Blocks, Merck or Fluka and were used without purification. Column chromatography (CC) was performed on silica gel (70-200 μm) coated with silica, preparative thin layer chromatography (TLC) on 1 mm SIL G-100 UV_{254} glass plates (Macherey-Nagel) and reaction progress was monitored by TLC on Alugram SIL G UV_{254} (Macherey-Nagel). 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) and CD_3COCD_3: δ = 2.05 ppm (^{1}H-NMR) and δ = 30.8 ppm (^{13}C-NMR)). Signals are described as s, d, t, dd, ddd, m, dt, td, q for singlet, doublet, triplet, doublet of doublets, doublet of doublets of doublets, multiplet, doublet of triplets, triplet of doublets and quadruplet respectively. All coupling constants (*J*) are given in hertz (Hz). Mass spectra (ESI) were recorded on a TSQ Quantum (Thermo Finnigan) instrument.
Tested compounds are > 98 % 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.

General procedure for Suzuki coupling

Method A: A mixture of 2-bromothiophene derivative (1 eq), boronic acid derivative (1.2 eq), caesium carbonate (4 eq) and tetrakis(triphenylphosphine) palladium (0.01 eq) in an oxygen free DME/water (1:1) solution was refluxed under nitrogen atmosphere for 2 h (method A1) or 4 h (method A2), respectively. The reaction mixture was cooled to rt. 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 column chromatography (CC), CC followed by preparative TLC or CC followed by preparative HPLC, respectively.

General procedure for ether cleavage

Method B: To a solution of methoxybenzene derivative (1 eq) in dry dichloromethane at -78 °C (dry ice/acetone bath), boron tribromide in dichloromethane (1 M, 3 eq per methoxy function) was added dropwise. The reaction mixture was stirred for 20 h at rt 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 sodium sulfate, filtered and concentrated to dryness. The product was purified by CC, CC followed by preparative HPLC or recrystallization, respectively.

General procedure for purification using preparative HPLC

All declared compounds were purified via an Agilent Technologies Series 1200–preparative HPLC using a RP C18 Nucleodur 100-5 column (30 x 100 mm / 50 µM from Macherey Nagel GmbH) as stationary phase with a linear gradient run (solvents: acetonitrile, water) starting from 20 % acetonitrile up to 100 % in 36 min.

Experimental and spectroscopic data of all compounds

(5-Bromo-2-thienyl)(3-methoxyphenyl)methanone (**1b**). A mixture of 2-bromothiophene (145 mg, 0.89 mmol), 3-methoxybenzoyl chloride (152 mg, 0.89 mmol) and aluminium chloride (119 mg, 0.89 mmol) in anhydrous dichloromethane was stirred at 0 °C for 0.5 h. The reaction mixture was warmed to rt and stirred for 1 h. HCl 1M 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 (hexane/ethyl acetate 97:3); yield: 77 % (204 mg, white powder). ^{1}H NMR (CD_3COCD_3): 7.55 (d, J = 4.1 Hz, 1H), 7.47 (t, J = 7.9 Hz, 1H), 7.42 (td, J = 1.5 Hz and J = 7.9 Hz, 1H), 7.35-7.34 (m, 1H), 7.33 (d, J = 4.1 Hz, 1H), 7.22 (ddd, J = 0.9 Hz and J = 2.5 Hz and J = 8.2 Hz, 1H), 3.88 (s, 3H); ^{13}C NMR (CD_3COCD_3): 187.90, 161.75, 140.45, 137.40, 133.85, 131.60, 123.95, 123.05, 120.45, 115.45, 56.85.

(5-Bromo-2-thienyl)(3-hydroxyphenyl)methanone (**1a**). The title compound was prepared by reaction of (5-bromo-2-thienyl)(3-methoxyphenyl)methanone (**1b**) (650 mg, 2.19 mmol) and boron tribromide (6.57 mmol) according to method B. The product was used in the next step without further purification; quantitative yield (620 mg, white powder). ^{1}H NMR (CD_3COCD_3): 8.76 (s, 1H), 7.54 (d, J = 4.1 Hz, 1H), 7.39 (t, J = 7.8 Hz, 1H), 7.34-7.32 (m, 2H), 7.30 (t, J = 2.1 Hz, 1H), 7.13 (ddd, J = 1.3 Hz and J = 2.5 Hz and J = 8.2 Hz, 1H); ^{13}C NMR (CD_3COCD_3): 187.90, 159.45, 147.25, 140.45, 137.25, 133.80, 131.70, 123.80, 122.10, 121.60, 117.25.

(3-Hydroxyphenyl)(5-phenyl-2-thienyl)methanone (**1**). The title compound was prepared by reaction of (5-bromo-2-thienyl)(3-hydroxyphenyl)methanone (**1a**) (150 mg, 0.53 mmol), benzene boronic acid (78 mg, 0.64 mmol), caesium carbonate (691 mg, 2.12 mmol) and tetrakis(triphenylphosphine) palladium (6 mg, 5 μmol) according to method A1. The product was purified by CC (hexane/ethyl acetate 8:2); yield: 28 % (42 mg, beige powder); ^{1}H NMR (CD_3COCD_3): 8.81 (s, 1H), 7.81-7.80 (m, 2H), 7.72 (d, J = 4.1 Hz, 1H), 7.60 (d, J = 4.1 Hz, 1H), 7.49 (t, J = 7.9 Hz, 2H), 7.44-7.39 (m, 2H), 7.37 (dt, J = 1.5 Hz and J = 7.6 Hz, 1H), 7.34-7.33 (m, 1H), 7.13 (ddd, J = 1.5 Hz and J = 2.5 Hz and J = 7.9 Hz, 1H); ^{13}C NMR (CD_3COCD_3): 188.75, 159.40, 154.30, 144.20, 141.30, 137.85, 135.15, 131.55, 131.15, 131.05, 128.05, 126.30, 122.05, 121.25, 117.30; MS (ESI): 281 $(M+H)^+$.

(3-Methoxyphenyl)[5-(2-methoxyphenyl)-2-thienyl]methanone (**2a**). A mixture of (5-bromo-2-thienyl)(3-methoxyphenyl)methanone (**1b**) (200 mg, 0.67 mmol), 2-methoxybenzene boronic acid (122 mg, 0.80 mmol), caesium carbonate (873 mg, 2.68 mmol) and tetrakis(triphenylphosphine) palladium (8 mg, 7 μmol) was suspended in an oxygen free

DME/EtOH/water (1:1:1) solution. The reaction mixture was exposed to microwave irradiation (15 min, 150 W, 150 °C, 15 bar). After reaching rt, water was added 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 (hexane/ethyl acetate 9:1); yield: 67 % (145 mg, yellow powder). ^{1}H NMR (CD_3COCD_3): 7.85 (dd, J = 1.9 Hz and J = 7.9 Hz, 2H), 7.69 (d, J = 1.3 Hz, 1H), 7.49-7.44 (m, 2H), 7.42-7.38 (m, 2H), 7.21-7.18 (m, 2H), 7.07 (dt, J = 0.9 Hz and J = 7.9 Hz, 1H), 4.01 (s, 3H), 3.89 (s, 3H); ^{13}C NMR (CD_3COCD_3): 189.10, 161.70, 158.10, 149.85, 144.05, 141.70, 136.50, 132.15, 131.45, 130.15, 127.80, 123.75, 123.10, 122.95, 119.90, 115.50, 114.05, 57.10, 56.80.

(3-Hydroxyphenyl)[5-(2-methoxyphenyl)-2-thienyl]methanone (**2**). The title compound was obtained by reaction of (3-methoxyphenyl)[5-(2-methoxyphenyl)-2-thienyl]methanone (**2a**) (145 mg, 0.45 mmol) and boron tribromide (2.70 mmol) according to method B. The product was purified by CC (hexane/ethyl acetate 8:2) followed by preparative HPLC; yield: 55 % (76 mg, yellow oil); ^{1}H NMR (CD_3COCD_3): 7.83 (dd, J = 1.6 Hz and J = 7.9 Hz, 1H), 7.69 (d, J = 4.1 Hz, 1H), 7.67 (d, J = 4.1 Hz, 1H), 7.41-7.35 (m, 4H), 7.18 (d, J = 7.9 Hz, 1H), 7.14-7.12 (m, 1H), 7.07-7.04 (m, 1H), 4.00 (s, 3H); ^{13}C NMR (CD_3COCD_3): 189.20, 159.30, 158.05, 149.70, 144.10, 141.70, 136.40, 132.10, 131.45, 130.10, 127.70, 123.75, 122.90, 122.05, 120.95, 117.25, 114.00, 57.10; MS (ESI): 311 $(M+H)^+$.

(3-Hydroxyphenyl)[5-(3-methoxyphenyl)-2-thienyl]methanone (**3**). The title compound was prepared by reaction of (5-bromo-2-thienyl)(3-hydroxyphenyl)methanone (**1a**) (150 mg, 0.53 mmol), 3-methoxybenzene boronic acid (97 mg, 0.64 mmol), caesium carbonate (691 mg, 2.12 mmol) and tetrakis(triphenylphosphine) palladium (6 mg, 5 µmol) according to method A1. The product was purified by CC (hexane/ethyl acetate 9:1); yield: 94 % (154 mg, beige powder); ^{1}H NMR (CD_3COCD_3): 7.71 (d, J = 4.1 Hz, 1H), 7.60 (d, J = 4.1 Hz, 1H), 7.42-7.35 (m, 4H), 7.34 (t, J = 1.9 Hz, 1H), 7.32 (t, J = 1.9 Hz, 1H), 7.13 (ddd, J = 1.3 Hz and J = 2.2 Hz and J = 7.6 Hz, 1H), 7.00 (ddd, J = 1.3 Hz and J = 2.2 Hz and J = 7.6 Hz, 1H), 3.89 (s, 3H); ^{13}C NMR (CD_3COCD_3): 188.75, 162.35, 159.45, 154.20, 144.25, 141.30, 137.75, 136.45, 132.25, 131.60, 126.55, 122.10, 121.25, 120.45, 117.30, 116.75, 113.35, 56.80; MS (ESI): 311 $(M+H)^+$.

(3-Hydroxyphenyl)[5-(4-methoxyphenyl)-2-thienyl]methanone (**4**). The title compound was prepared by reaction of (5-bromo-2-thienyl)(3-hydroxyphenyl)methanone (**1a**) (150 mg, 0.53 mmol), 4-methoxybenzene boronic acid (97 mg, 0.64 mmol), caesium carbonate (691 mg, 2.12 mmol) and tetrakis(triphenylphosphine) palladium (6 mg, 5 µmol) according to method A1. The product was purified by CC (hexane/ethyl acetate 8:2) followed by preparative TLC (hexane/ethyl acetate 7:3); yield: 96 % (158 mg, yellow powder); ^{1}H NMR (CD_3COCD_3): 8.72

(s, 1H), 7.72 (d, *J* = 8.8 Hz, 2H), 7.66 (d, *J* = 4.1 Hz, 1H), 7.45 (d, *J* = 4.1 Hz, 1H), 7.39 (t, *J* = 7.8 Hz, 1H), 7.35 (dt, *J* = 1.3 Hz and *J* = 7.8 Hz, 1H), 7.34 (t, *J* = 2.1 Hz, 1H), 7.12 (ddd, *J* = 1.3 Hz and *J* = 2.5 Hz and *J* = 7.9 Hz, 1H), 7.02 (d, *J* = 8.8 Hz, 2H), 3.85 (s, 3H); ^{13}C NMR (CD_3COCD_3): 188.60, 162.65, 159.35, 154.65, 143.15, 141.45, 138.00, 131.50, 129.45, 127.75, 125.05, 122.05, 121.05, 117.25, 116.50, 56.80; MS (ESI): 311 $(M+H)^+$.

(3-Hydroxyphenyl)[5-(6-methoxypyridin-3-yl)-2-thienyl]methanone (**5**). The title compound was prepared by reaction of (5-bromo-2-thienyl)(3-hydroxyphenyl)methanone (**1a**) (150 mg, 0.53 mmol), 6-methoxypyridine-3-boronic acid (98 mg, 0.64 mmol), caesium carbonate (691 mg, 2.12 mmol) and tetrakis(triphenylphosphine) palladium (6 mg, 5 µmol) according to method A1. The product was purified by CC (hexane/ethyl acetate 85:15); yield: 63 % (104 mg, beige powder); ^{1}H NMR (CD_3COCD_3): 8.86 (s, 1H), 8.60 (dd, *J* = 0.6 Hz and *J* = 2.4 Hz, 1H), 8.06 (dd, *J* = 2.5 Hz and *J* = 8.5 Hz, 1H), 7.71 (d, *J* = 4.1 Hz, 1H), 7.55 (d, *J* = 4.1 Hz, 1H), 7.40 (t, *J* = 7.8 Hz, 1H), 7.36 (dt, *J* = 1.3 Hz and *J* = 7.6 Hz, 1H), 7.33-7.32 (m, 1H), 7.12 (ddd, *J* = 1.3 Hz and *J* = 2.5 Hz and *J* = 7.8 Hz, 1H), 6.88 (dd, *J* = 0.6 Hz and *J* = 8.5 Hz, 1H), 3.94 (s, 3H); ^{13}C NMR (CD_3COCD_3): 188.65, 166.40, 159.45, 150.95, 146.40, 141.25, 138.55, 137.90, 131.55, 126.10, 122.05, 121.25, 117.25, 113.05, 54.95; MS (ESI): 312 $(M+H)^+$.

[5-(3,4-Dimethoxyphenyl)-2-thienyl](3-hydroxyphenyl)methanone (**6**). The title compound was prepared by reaction of (5-bromo-2-thienyl)(3-hydroxyphenyl)methanone (**1a**) (150 mg, 0.53 mmol), 3,4-dimethoxybenzene boronic acid (117 mg, 0.64 mmol), caesium carbonate (691 mg, 2.12 mmol) and tetrakis(triphenylphosphine) palladium (6 mg, 5 µmol) according to method A1. The product was purified by CC (hexane/ethyl acetate 8:2); yield: 73 % (132 mg, yellow powder); ^{1}H NMR (CD_3COCD_3): 8.78 (s, 1H), 7.68 (d, *J* = 4.1 Hz, 1H), 7.51 (d, *J* = 4.1 Hz, 1H), 7.39 (t, *J* = 7.8 Hz, 1H), 7.38-7.34 (m, 3H), 7.32-7.31 (m, 1H), 7.12 (ddd, *J* = 1.3 Hz and *J* = 2.5 Hz and *J* = 7.9 Hz, 1H), 7.06-7.04 (m, 1H), 3.92 (s, 3H), 3.87 (s, 3H); ^{13}C NMR (CD_3COCD_3): 189.65, 159.40, 152.60, 151.80, 141.50, 137.95, 131.55, 128.05, 125.35, 122.00, 121.10, 120.95, 117.25, 114.05, 111.70, 57.25, 57.20; MS (ESI): 341 $(M+H)^+$.

(3-Hydroxyphenyl)[5-(3,4,5-trimethoxyphenyl)-2-thienyl]methanone (**7**). The title compound was prepared by reaction of (5-bromo-2-thienyl)(3-hydroxyphenyl)methanone (**1a**) (150 mg, 0.53 mmol), 3,4,5-trimethoxybenzene boronic acid (136 mg, 0.64 mmol), caesium carbonate (691 mg, 2.12 mmol) and tetrakis(triphenylphosphine) palladium (6 mg, 5 µmol) according to method A1. The product was purified by CC (hexane/ethyl acetate 8:2); yield: 71 % (140 mg, yellow powder); ^{1}H NMR (CD_3COCD_3): 8.76 (s, 1H), 7.68 (d, *J* = 4.1 Hz, 1H), 7.56 (d, *J* = 4.1 Hz, 1H), 7.40 (t, *J* = 7.8 Hz, 1H), 7.35 (dt, *J* = 1.3 Hz and *J* = 7.6 Hz, 1H), 7.33-7.32 (m, 1H), 7.12 (ddd, *J* = 1.3 Hz and *J* = 2.5 Hz and *J* = 7.9 Hz, 1H), 7.07 (s, 2H), 3.93 (s,

6H), 3.78 (s, 3H); ^{13}C NMR (CD_3COCD_3): 188.70, 159.40, 155.95, 154.75, 143.80, 141.35, 137.75, 131.55, 130.70, 126.20, 122.05, 121.15, 117.25, 105.85, 61.70, 57.65; MS (ESI): 371 $(M+H)^+$.

(3-Hydroxyphenyl)[5-(2,3,4-trimethoxyphenyl)-2-thienyl]methanone (**8**). The title compound was prepared by reaction of (5-bromo-2-thienyl)(3-hydroxyphenyl)methanone (**1a**) (150 mg, 0.53 mmol), 2,3,4-trimethoxybenzene boronic acid (136 mg, 0.64 mmol), caesium carbonate (691 mg, 2.12 mmol) and tetrakis(triphenylphosphine) palladium (6 mg, 5 µmol) according to method A1. The product was purified by CC (hexane/ethyl acetate 8:2); yield: 62 % (122 mg, yellow powder); ^{1}H NMR (CD_3COCD_3): 8.80 (s, 1H), 7.66 (d, *J* = 4.1 Hz, 1H), 7.59-7.56 (m, 2H) 7.39 (t, *J* = 7.8 Hz, 1H), 7.35 (dt, *J* = 1.3 Hz and *J* = 7.6 Hz, 1H), 7.33-7.32 (m, 1H), 7.11 (ddd, *J* = 1.3 Hz and *J* = 2.5 Hz and *J* = 7.9 Hz, 1H), 6.92 (d, *J* = 9.1 Hz, 1H), 3.95 (s, 3H), 3.91 (s, 3H), 3.87 (s, 3H); ^{13}C NMR (CD_3COCD_3): 189.10, 159.40, 156.60, 152.85, 149.65, 143.70, 141.80, 136.60, 131.45, 126.65, 124.40, 122.00, 121.65, 121.00, 117.25, 110.35, 62.05, 62.00, 57.50; MS (ESI): 371 $(M+H)^+$.

5-(2-Ethoxyphenyl)-2-thienyl](3-hydroxyphenyl)methanone (**9**). The title compound was prepared by reaction of (5-bromo-2-thienyl)(3-hydroxyphenyl)methanone (**1a**) (150 mg, 0.53 mmol), 2-ethoxybenzene boronic acid (106 mg, 0.64 mmol), caesium carbonate (691 mg, 2.12 mmol) and tetrakis(triphenylphosphine) palladium (6 mg, 5 µmol) according to method A1. The product was purified by CC (hexane/ethyl acetate 85:15); yield: 88 % (151 mg, yellow oil); ^{1}H NMR (CD_3COCD_3): 7.87 (dd, *J* = 1.5 Hz and *J* = 7.9 Hz, 1H), 7.74 (d, *J* = 4.1 Hz, 1H), 7.69 (d, *J* = 4.1 Hz, 1H), 7.41-7.35 (m, 3H), 7.34 (t, *J* = 2.1 Hz, 1H), 7.18-7.16 (m, 1H), 7.12 (ddd, *J* = 1.3 Hz and *J* = 2.5 Hz and *J* = 7.6 Hz, 1H), 7.06 (td, *J* = 1.3 Hz and *J* = 7.6 Hz, 1H), 4.28 (q, *J* = 6.9 Hz, 2H), 1.55 (t, *J* = 6.9 Hz, 3H); ^{13}C NMR (CD_3COCD_3): 189.15, 157.30, 144.00, 141.75, 138.40, 136.30, 132.05, 131.45, 130.05, 127.55, 123.80, 122.80, 122.10, 121.00, 117.30, 114.75, 113.00, 66.35, 16.10; MS (ESI): 325 $(M+H)^+$.

[5-(3-Ethoxyphenyl)-2-thienyl](3-hydroxyphenyl)methanone (**10**). The title compound was prepared by reaction of (5-bromo-2-thienyl)(3-hydroxyphenyl)methanone (**1a**) (150 mg, 0.53 mmol), 3-ethoxybenzene boronic acid (106 mg, 0.64 mmol), caesium carbonate (691 mg, 2.12 mmol) and tetrakis(triphenylphosphine) palladium (6 mg, 5 µmol) according to method A1. The product was purified by CC (hexane/ethyl acetate 8:2) followed by preparative TLC (hexane/ethyl acetate 7:3); yield: 91 % (157 mg, beige powder); ^{1}H NMR (CD_3COCD_3): 8.80 (s, 1H), 7.69 (d, *J* = 4.1 Hz, 1H), 7.56 (d, *J* = 4.1 Hz, 1H), 7.41-7.34 (m, 4H), 7.32 (dt, *J* = 1.3 Hz and *J* = 7.6 Hz, 1H), 7.28 (t, *J* = 2.1 Hz, 1H), 7.13 (ddd, *J* = 1.3 Hz and *J* = 2.5 Hz and *J* = 7.6 Hz, 1H), 6.96 (ddd, *J* = 1.3 Hz and *J* = 2.5 Hz and *J* = 7.9 Hz, 1H), 4.11 (q, *J* = 6.9 Hz, 2H),

1.38 (t, *J* = 6.9 Hz, 3H); ^{13}C NMR (CD_3COCD_3): 188.70, 161.55, 159.35, 154.20, 144.10, 141.25, 137.70, 136.35, 132.20, 131.55, 126.40, 122.05, 121.20, 120.25, 117.25, 117.15, 113.90, 65.25, 16.05; MS (ESI): 325 $(M+H)^+$.

[5-(4-Ethoxyphenyl)-2-thienyl](3-hydroxyphenyl)methanone (**11**). The title compound was prepared by reaction of (5-bromo-2-thienyl)(3-hydroxyphenyl)methanone (**1a**) (150 mg, 0.53 mmol), 4-ethoxybenzene boronic acid (106 mg, 0.64 mmol), caesium carbonate (691 mg, 2.12 mmol) and tetrakis(triphenylphosphine) palladium (6 mg, 5 µmol) according to method A1. The product was purified by CC (hexane/ethyl acetate 8:2); yield: 71 % (122 mg, beige powder); ^{1}H NMR (CD_3COCD_3): 8.76 (s, 1H), 7.72 (d, *J* = 8.8 Hz, 2H), 7.67 (d, *J* = 4.1 Hz, 1H), 7.45 (d, *J* = 4.1 Hz, 1H), 7.39 (t, *J* = 7.8 Hz, 1H), 7.35 (dt, *J* = 1.3 Hz and *J* = 7.8 Hz, 1H), 7.33-7.32 (m, 1H), 7.11 (ddd, *J* = 0.9 Hz and *J* = 2.5 Hz and *J* = 7.8 Hz, 1H), 7.01 (d, *J* = 8.8 Hz, 2H), 4.11 (q, *J* = 6.9 Hz, 2H), 1.38 (t, *J* = 6.9 Hz, 3H); ^{13}C NMR (CD_3COCD_3): 188.60, 162.00, 159.40, 154.75, 143.10, 141.45, 138.05, 131.50, 129.45, 127.60, 125.00, 122.00, 121.05, 117.25, 117.00, 65.30, 16.00; MS (ESI): 325 $(M+H)^+$.

{5-[3-(Benzyloxy)phenyl]-2-thienyl}(3-hydroxyphenyl)methanone (**12**). The title compound was prepared by reaction of (5-bromo-2-thienyl)(3-hydroxyphenyl)methanone (**1a**) (150 mg, 0.53 mmol), 3-(benzyloxy)benzene boronic acid (146 mg, 0.64 mmol), caesium carbonate (691 mg, 2.12 mmol) and tetrakis(triphenylphosphine) palladium (6 mg, 5 µmol) according to method A1. The product was purified by CC (hexane/ethyl acetate 8:2); yield: 71 % (146 mg, beige powder); ^{1}H NMR (CD_3COCD_3): 8.76 (s, 1H), 7.70 (d, *J* = 4.1 Hz, 1H), 7.58 (d, *J* = 4.1 Hz, 1H), 7.52-7.51 (m, 2H), 7.43-7.32 (m, 9H), 7.13 (ddd, *J* = 1.3 Hz and *J* = 2.5 Hz and *J* = 7.6 Hz, 1H), 7.08 (ddd, *J* = 1.3 Hz and *J* = 2.5 Hz and *J* = 7.6 Hz, 1H), 5.22 (s, 2H); ^{13}C NMR (CD_3COCD_3): 188.70, 161.40, 159.40, 154.10, 144.20, 141.25, 139.10, 137.75, 136.45, 132.30, 131.55, 130.30, 129.70, 129.50, 126.50, 122.10, 121.25, 120.65, 117.65, 117.30, 114.35, 71.65; MS (ESI): 387 $(M+H)^+$.

(3-Hydroxyphenyl)(5-{3-[(2-methoxybenzyl)oxy]phenyl}-2-thienyl)methanone (**13**). The title compound was prepared by reaction of (5-bromo-2-thienyl)(3-hydroxyphenyl)methanone (**1a**) (150 mg, 0.53 mmol), 3-[(2-methoxybenzyl)oxy]benzene boronic acid (165 mg, 0.64 mmol), caesium carbonate (691 mg, 2.12 mmol) and tetrakis(triphenylphosphine) palladium (6 mg, 5 µmol) according to method A1. The product was purified by CC (hexane/ethyl acetate 7:3); yield: 64 % (142 mg, beige powder); ^{1}H NMR (CD_3COCD_3): 8.74 (s, 1H), 7.71 (d, *J* = 4.1 Hz, 1H), 7.60 (d, *J* = 4.1 Hz, 1H), 7.48 (m, 1H), 7.42-7.36 (m, 5H), 7.34-7.31 (m, 2H), 7.13 (ddd, *J* = 1.3 Hz and *J* = 2.5 Hz and *J* = 7.9 Hz, 1H), 7.08-7.04 (m, 2H), 6.97 (td, *J* = 1.3 Hz and *J* = 7.6 Hz, 1H), 5.21 (s, 2H), 3.90 (s, 3H); ^{13}C NMR (CD_3COCD_3): 188.75, 161.55, 159.40,

159.15, 154.20, 144.20, 141.30, 137.75, 136.45, 132.30, 131.60, 131.15, 130.85, 126.95, 126.50, 122.30, 122.10, 121.25, 120.55, 117.60, 117.30, 114.25, 112.55, 66.70, 56.95; MS (ESI): 417 $(M+H)^+$.

(3-Hydroxyphenyl)(5-{3-[(3-methoxybenzyl)oxy]phenyl}-2-thienyl)methanone (**14**). The title compound was prepared by reaction of (5-bromo-2-thienyl)(3-hydroxyphenyl)methanone (**1a**) (150 mg, 0.53 mmol), 3-[(3-methoxybenzyl)oxy]benzene boronic acid (165 mg, 0.64 mmol), caesium carbonate (691 mg, 2.12 mmol) and tetrakis(triphenylphosphine) palladium (6 mg, 5 μmol) according to method A1. The product was purified by CC (hexane/ethyl acetate 8:2); yield: 74 % (163 mg, beige powder); ^{1}H NMR (CD_3COCD_3): 8.75 (s, 1H), 7.71 (d, J = 4.1 Hz, 1H), 7.60 (d, J = 4.1 Hz, 1H), 7.44-7.36 (m, 5H), 7.34-7.30 (m, 2H), 7.13 (ddd, J = 1.3 Hz and J = 2.5 Hz and J = 7.9 Hz, 1H), 7.10-7.08 (m, 3H), 6.91-6.89 (m, 1H), 5.21 (s, 2H), 3.81 (s, 3H); ^{13}C NMR (CD_3COCD_3): 188.75, 161.95, 161.40, 159.45, 154.10, 141.30, 140.70, 137.75, 136.50, 132.30, 131.60, 131.40, 126.55, 122.10, 121.55, 121.25, 120.70, 117.70, 117.30, 115.15, 115.00, 114.40, 71.55, 56.55; MS (ESI): 417 $(M+H)^+$.

(3-Hydroxyphenyl)(5-{3-[(4-methoxybenzyl)oxy]phenyl}-2-thienyl)methanone (**15**). The title compound was prepared by reaction of (5-bromo-2-thienyl)(3-hydroxyphenyl)methanone (**1a**) (150 mg, 0.53 mmol), 3-[(4-methoxybenzyl)oxy]benzene boronic acid (165 mg, 0.64 mmol), caesium carbonate (691 mg, 2.12 mmol) and tetrakis(triphenylphosphine) palladium (6 mg, 5 μmol) according to method A1. The product was purified by CC (hexane/ethyl acetate 8:2); yield: 59 % (131 mg, beige powder); ^{1}H NMR (CD_3COCD_3): 8.83 (s, 1H), 7.71 (d, J = 4.1 Hz, 1H), 7.60 (d, J = 4.1 Hz, 1H), 7.44 (d, J = 8.5 Hz, 2H), 7.41-7.36 (m, 5H), 7.34-7.33 (m, 1H), 7.13 (ddd, J = 1.3 Hz and J = 2.5 Hz and J = 7.9 Hz, 1H), 7.07 (ddd, J = 1.3 Hz and J = 2.5 Hz and J = 7.9 Hz, 1H), 6.96 (d, J = 8.5 Hz, 2H), 5.14 (s, 2H), 3.80 (s, 3H); ^{13}C NMR (CD_3COCD_3): 186.90, 181.65, 161.60, 161.50, 159.40, 144.25, 141.30, 137.75, 136.45, 132.30, 131.60, 131.30, 131.00, 126.50, 122.10, 121.25, 120.55, 117.75, 117.30, 115.75, 114.35, 71.50, 56.55; MS (ESI): 417 $(M+H)^+$.

(3-Hydroxyphenyl)(5-{3-[(3,5-dimethoxybenzyl)oxy]phenyl}-2-thienyl)methanone (**16**). The title compound was prepared by reaction of (5-bromo-2-thienyl)(3-hydroxyphenyl)methanone (**1a**) (150 mg, 0.53 mmol), 3-[(3,5-dimethoxybenzyl)oxy]benzene boronic acid (184 mg, 0.64 mmol), caesium carbonate (691 mg, 2.12 mmol) and tetrakis(triphenylphosphine) palladium (6 mg, 5 μmol) according to method A1. The product was purified by CC (hexane/ethyl acetate 8:2) followed by preparative HPLC; yield: 47 % (112 mg, beige powder); ^{1}H NMR (CD_3COCD_3): 7.71 (d, J = 4.1 Hz, 1H), 7.59 (d, J = 4.1 Hz, 1H), 7.42-7.35 (m, 5H), 7.33-7.32 (m, 1H), 7.12 (ddd, J = 1.3 Hz and J = 2.5 Hz and J = 7.9 Hz, 1H),

7.09-7.06 (m, 1H), 6.68 (d, *J* = 2.2 Hz, 2H), 6.44 (t, *J* = 2.2 Hz, 1H), 5.17 (s, 2H), 3.79 (s, 6H); ^{13}C NMR (CD_3COCD_3): 188.80, 163.10, 161.35, 159.50, 154.10, 144.25, 141.50, 141.25, 137.75, 136.45, 132.30, 131.55, 126.55, 122.00, 121.25, 120.65, 117.70, 117.30, 114.40, 107.20, 101.35, 71.55, 56.65; MS (ESI): 447 $(M+H)^+$.

(3-Hydroxyphenyl)(5-{3-[(2-chlorobenzyl)oxy]phenyl}-2-thienyl)methanone (**17**). The title compound was prepared by reaction of (5-bromo-2-thienyl)(3-hydroxyphenyl)methanone (**1a**) (150 mg, 0.53 mmol), 3-[(2-chlorobenzyl)oxy]benzene boronic acid (168 mg, 0.64 mmol), caesium carbonate (691 mg, 2.12 mmol) and tetrakis(triphenylphosphine) palladium (6 mg, 5 µmol) according to method A1. The product was purified by CC (hexane/ethyl acetate 8:2); yield: 51 % (113 mg, beige powder); ^{1}H NMR (CD_3COCD_3): 8.78 (s, 1H), 7.71 (d, *J* = 4.1 Hz, 1H), 7.68-7.66 (m, 1H), 7.61 (d, *J* = 4.1 Hz, 1H), 7.50-7.48 (m, 1H), 7.46-7.45 (m, 1H), 7.43-7.36 (m, 6H), 7.34-7.33 (m, 1H), 7.14-7.10 (m, 2H), 5.31 (s, 2H); ^{13}C NMR (CD_3COCD_3): 188.75, 161.20, 159.40, 153.95, 144.30, 141.25, 137.75, 136.60, 136.55, 134.80, 132.40, 131.70, 131.55, 131.50, 131.30, 129.15, 126.65, 122.10, 121.25, 121.00, 117.55, 117.30, 114.40, 69.10; MS (ESI): 421 $(M+H)^+$.

(3-Hydroxyphenyl)(5-{3-[(3-chlorobenzyl)oxy]phenyl}-2-thienyl)methanone (**18**). The title compound was prepared by reaction of (5-bromo-2-thienyl)(3-hydroxyphenyl)methanone (**1a**) (150 mg, 0.53 mmol), 3-[(3-chlorobenzyl)oxy]benzene boronic acid (168 mg, 0.64 mmol), caesium carbonate (691 mg, 2.12 mmol) and tetrakis(triphenylphosphine) palladium (6 mg, 5 µmol) according to method A1. The product was purified by CC (hexane/ethyl acetate 8:2); yield: 55 % (122 mg, beige powder); ^{1}H NMR (CD_3COCD_3): 8.80 (s, 1H), 7.70 (d, *J* = 4.1 Hz, 1H), 7.58 (d, *J* = 4.1 Hz, 1H), 7.57-7.56 (m, 1H), 7.48-7.46 (m, 1H), 7.43-7.34 (m, 8H), 7.13 (ddd, *J* = 1.3 Hz and *J* = 2.5 Hz and *J* = 7.9 Hz, 1H), 7.10-7.08 (m, 1H), 5.24 (s, 2H); ^{13}C NMR (CD_3COCD_3): 188.75, 161.10, 159.40, 153.95, 144.25, 141.65, 141.25, 137.70, 136.50, 135.75, 132.35, 132.05, 131.55, 129.70, 129.25, 127.75, 126.55, 122.05, 121.25, 120.85, 117.60, 117.30, 114.35, 70.70; MS (ESI): 421 $(M+H)^+$.

(3-Hydroxyphenyl)(5-{3-[(4-chlorobenzyl)oxy]phenyl}-2-thienyl)methanone (**19**). The title compound was prepared by reaction of (5-bromo-2-thienyl)(3-hydroxyphenyl)methanone (**1a**) (150 mg, 0.53 mmol), 3-[(4-chlorobenzyl)oxy]benzene boronic acid (168 mg, 0.64 mmol), caesium carbonate (691 mg, 2.12 mmol) and tetrakis(triphenylphosphine) palladium (6 mg, 5 µmol) according to method A1. The product was purified by CC (hexane/ethyl acetate 8:2); yield: 63 % (141 mg, beige powder); ^{1}H NMR (CD_3COCD_3): 8.76 (s, 1H), 7.71 (d, *J* = 4.1 Hz, 1H), 7.60 (d, *J* = 4.1 Hz, 1H), 7.54 (d, *J* = 8.8 Hz, 2H), 7.45-7.38 (m, 6H), 7.37 (dt, *J* = 1.3 Hz and *J* = 7.6 Hz, 1H), 7.34-7.33 (m, 1H), 7.13 (ddd, *J* = 1.3 Hz and *J* = 2.5 Hz and *J* = 7.9 Hz,

1H), 7.09-7.08 (m, 1H), 5.24 (s, 2H); ^{13}C NMR (CD_3COCD_3): 188.75, 161.20, 159.40, 154.00, 144.30, 141.25, 138.15, 137.75, 136.50, 135.05, 132.35, 131.55, 131.20, 130.40, 126.55, 122.10, 121.25, 120.85, 117.65, 117.30, 114.35, 70.85; MS (ESI): 421 $(M+H)^+$.

3-[5-(3-Methoxybenzoyl)-2-thienyl]benzonitrile (**20a**). The title compound was prepared by reaction of (5-bromo-2-thienyl)(3-methoxyphenyl)methanone (**1b**) (300 mg, 1.01 mmol), 3-cyanobenzene boronic acid (178 mg, 1.21 mmol), caesium carbonate (1.32 g, 4.04 mmol) and tetrakis(triphenylphosphine) palladium (12 mg, 10 μmol) according to method A2. The product was purified by CC (hexane/ethyl acetate 9:1); yield: 88 % (282 mg, beige powder); ^{1}H NMR ($CDCl_3$): 7.95 (t, J = 1.6 Hz, 1H), 7.90-7.88 (m, 1H), 7.67-7.64 (m, 2H), 7.55 (t, J = 7.9 Hz, 1H), 7.47 (dt, J = 1.3 Hz and J = 7.6 Hz, 1H), 7.42 (t, J = 7.9 Hz, 1H), 7.40-7.39 (m, 2H), 7.15 (ddd, J = 1.3 Hz and J = 2.8 Hz and J = 8.2 Hz, 1H), 3.88 (s, 3H); ^{13}C NMR ($CDCl_3$): 187.50, 159.70, 149.70, 138.95, 135.65, 134.70, 132.05, 130.45, 130.10, 129.55, 129.50, 125.00, 121.65, 118.80, 118.15, 113.80, 113.60, 55.50.

3-[5-(3-Hydroxybenzoyl)-2-thienyl]benzonitrile (**20**). The title compound was prepared by reaction of 3-[5-(3-methoxybenzoyl)-2-thienyl]benzonitrile (**20a**) (282 mg, 0.88 mmol) and boron tribromide (2.64 mmol) according to method B. The product was purified by recrystallization (EtOH/acetonitrile); yield: 66 % (176 mg, beige powder); ^{1}H NMR (CD_3COCD_3): 8.78 (s, 1H), 8.23 (t, J = 1.6 Hz, 1H), 8.12 (ddd, J = 0.9 Hz and J = 1.9 Hz and J = 7.9 Hz, 1H), 7.83-7.81 (m, 1H), 7.78-7.76 (m, 2H), 7.72 (td, J = 0.7 Hz and J = 7.9 Hz, 1H), 7.41 (t, J = 7.6 Hz, 1H), 7.40-7.38 (m, 1H), 7.36-7.35 (m, 1H), 7.14 (ddd, J = 1.6 Hz and J = 2.5 Hz and J = 7.6 Hz, 1H); ^{13}C NMR (CD_3COCD_3): 188.75, 151.25, 145.50, 141.00, 140.70, 137.75, 136.45, 134.15, 132.40, 131.65, 131.30, 127.85, 122.15, 121.45, 117.30, 115.35, 110.30; MS (ESI): 306 $(M+H)^+$.

***N*-{3-[5-(3-Methoxybenzoyl)-2-thienyl]phenyl}methanesulfonamide** (**21a**). The title compound was prepared by reaction of (5-bromo-2-thienyl)(3-methoxyphenyl)methanone (**1b**) (300 mg, 1.01 mmol), 3-[(methylsulfonyl)amino]benzene boronic acid (178 mg, 1.21 mmol), caesium carbonate (1.32 g, 4.04 mmol) and tetrakis(triphenylphosphine) palladium (12 mg, 10 μmol) according to method A2. The product was used in the next step without further purification; yield: 96 % (376 mg, beige powder); ^{1}H NMR (CD_3COCD_3): 7.76 (t, J = 1.9 Hz, 1H), 7.70 (d, J = 4.1 Hz, 1H), 7.55-7.52 (m, 2H), 7.48-7.43 (m, 3H), 7.41 (ddd, J = 0.9 Hz and J = 1.9 Hz and J = 7.9 Hz, 1H), 7.38-7.37 (m, 1H), 7.21-7.19 (m, 1H), 3.87 (s, 3H), 3.07 (s, 3H); ^{13}C NMR (CD_3COCD_3): 188.60, 161.60, 153.60, 144.30, 141.40, 141.05, 137.90, 136.15, 131.45, 126.70, 123.70, 123.05, 122.35, 120.05, 119.30, 115.50, 59.70, 40.65.

***N*-{3-[5-(3-Hydroxybenzoyl)-2-thienyl]phenyl}methanesulfonamide** (**21**). The title compound was prepared by reaction of *N*-{3-[5-(3-methoxybenzoyl)-2-thienyl]phenyl}methanesulfonamide (**21a**) (200 mg, 0.52 mmol) and boron tribromide (1.56 mmol) according to method B. The crude mixture was washed with MeOH. The resulted suspension was filtered and the precipitated product was evaporated to dryness.; yield: 87 % (168 mg, beige powder); ^{1}H NMR (CD_3COCD_3): 8.79 (s, 1H), 8.76 (s, 1H), 7.76 (t, *J* = 1.9 Hz, 1H), 7.73 (d, *J* = 4.1 Hz, 1H), 7.60-7.57 (m, 2H), 7.48 (t, *J* = 7.9 Hz, 1H), 7.42-7.36 (m, 3H), 7.34 (t, *J* = 1.9 Hz, 1H), 7.13 (ddd, *J* = 1.2 Hz and *J* = 2.5 Hz and *J* = 7.6 Hz, 1H), 3.07 (s, 3H); ^{13}C NMR (CD_3COCD_3): 188.75, 159.45, 153.50, 144.50, 141.45, 137.80, 132.30, 131.65, 126.70, 122.35, 122.10, 121.30, 119.30, 117.30, 40.65; MS (ESI): 374 $(M+H)^+$.

***N*-{3-[5-(3-Methoxyphenyl)-2-thienyl]benzyl}methanesulfonamide** (**22a**). The title compound was prepared by reaction of (5-bromo-2-thienyl)(3-methoxyphenyl)methanone (**1b**) (200 mg, 0.67 mmol), 3-{[(methylsulfonyl)amino]methyl}benzene boronic acid (183 mg, 0.80 mmol), caesium carbonate (1.32 g, 4.04 mmol) and tetrakis(triphenylphosphine) palladium (12 mg, 10 µmol) according to method A2. The product was purified by CC (hexane/ethyl acetate 9:1); yield: 94 % (253 mg, beige powder); ^{1}H NMR (CD_3COCD_3): 7.87-7.86 (m, 1H), 7.75-7.72 (m, 1H), 7.61 (d, *J* = 4.1 Hz, 1H), 7.51-7.45 (m, 4H), 7.39-7.38 (m, 1H), 7.22 (ddd, *J* = 1.6 Hz and *J* = 2.5 Hz and *J* = 7.6 Hz, 1H), 7.42 (s, 1H), 7.41 (s, 1H), 3.90 (s, 3H), 2.92 (s, 3H); ^{13}C NMR (CD_3COCD_3): 188.70, 161.75, 154.15, 144.20, 141.65, 138.00, 135.35, 131.55, 131.40, 130.55, 127.45, 127.05, 126.55, 123.05, 120.10, 115.50, 56.85, 48.30, 41.60.

***N*-{3-[5-(3-Hydroxybenzoyl)-2-thienyl]benzyl}methanesulfonamide** (**22**). The title compound was prepared by reaction of *N*-{3-[5-(3-methoxyphenyl)-2-thienyl]benzyl}methanesulfonamide (**22a**) (253 mg, 0.63 mmol) and boron tribromide (1.89 mmol) according to method B. The crude mixture was washed with MeOH. The resulted suspension was filtered and the precipitated product was evaporated to dryness.; yield: 81 % (197 mg, beige powder); ^{1}H NMR (CD_3COCD_3): 8.76 (s, 1H), 7.86 (s, 1H), 7.75-7.72 (m, 2H), 7.62 (d, *J* = 4.0 Hz, 4H), 7.50-7.48 (m, 2H), 7.41 (t, *J* = 7.8 Hz, 1H), 7.37 (dt, *J* = 1.4 Hz and *J* = 7.6 Hz, 1H), 7.34-7.33 (m, 1H), 7.13 (ddd, *J* = 1.2 Hz and *J* = 2.4 Hz and *J* = 7.6 Hz, 1H), 6.61 (s, 1H), 4.41 (s, 1H), 4.40 (s, 1H), 2.92 (s, 3H); ^{13}C NMR (CD_3COCD_3): 188.75, 159.45, 154.00, 144.30, 141.65, 141.25, 137.85, 135.35, 131.60, 131.40, 130.50, 127.45, 127.05, 126.50, 122.10, 121.25, 117.25, 48.30, 41.55; MS (ESI): 388 $(M+H)^+$.

***N*-{3-[5-(3-Hydroxybenzoyl)-2-thienyl]phenyl}-4-methylbenzenesulfonamide** (**23**). The title compound was prepared by reaction of (5-bromo-2-thienyl)(3-hydroxyphenyl)methanone (**1a**) (150 mg, 0.53 mmol), 3-{[(4-methylphenyl)sulfonyl]amino}benzene boronic acid (239 mg,

0.64 mmol), caesium carbonate (691 mg, 2.12 mmol) and tetrakis(triphenylphosphine) palladium (6 mg, 5 µmol) according to method A1. The crude mixture was washed with MeOH. The resulted suspension was filtered and the precipitated product was evaporated to dryness.; yield: 53 % (126 mg, beige powder); ^{1}H NMR (CD_3COCD_3): 7.75 (d, *J* = 8.2 Hz, 2H), 7.70 (d, *J* = 4.1 Hz, 1H), 7.62 (t, *J* = 1.8 Hz, 1H), 7.50-7.48 (m, 2H), 7.40 (t, *J* = 7.6 Hz, 1H), 7.38-7.32 (m, 5H), 7.27 (ddd, *J* = 0.9 Hz and *J* = 2.2 Hz and *J* = 8.2 Hz, 1H), 7.13 (ddd, *J* = 1.3 Hz and *J* = 2.5 Hz and *J* = 7.9 Hz, 1H), 2.35 (s, 3H); ^{13}C NMR (CD_3COCD_3): 188.70, 159.45, 153.40, 145.75, 144.45, 141.20, 140.95, 138.85, 137.80, 136.05, 132.10, 131.60, 131.55, 129.05, 126.60, 123.80, 122.70, 122.05, 121.30, 119.55, 117.30, 22.35; MS (ESI): 450 $(M+H)^+$.

(3-Hydroxyphenyl)[5-(2-naphthyl)-2-thienyl]methanone (**24**). The title compound was prepared by reaction of (5-bromo-2-thienyl)(3-hydroxyphenyl)methanone (**1a**) (150 mg, 0.53 mmol), 2-naphthaleneboronic acid (110 mg, 0.64 mmol), caesium carbonate (691 mg, 2.12 mmol) and tetrakis(triphenylphosphine) palladium (6 mg, 5 µmol) according to method A1. The product was purified by CC (hexane/ethyl acetate 8:2); yield: 57 % (100 mg, yellow powder); ^{1}H NMR (CD_3COCD_3): 8.86 (s, 1H), 8.36 (s, 1H), 8.03-8.00 (m, 2H), 7.95-7.91 (m, 2H), 7.76 (d, *J* = 4.1 Hz, 1H), 7.74 (d, *J* = 4.1 Hz, 1H), 7.59-7.54 (m, 2H), 7.42 (t, *J* = 7.6 Hz, 1H), 7.39 (dt, *J* = 1.5 Hz and *J* = 7.6 Hz, 1H), 7.37-7.36 (m, 1H), 7.15-7.13 (m, 1H); ^{13}C NMR (CD_3COCD_3): 188.75, 159.45, 154.30, 144.35, 141.30, 137.95, 135.55, 131.60, 130.90, 130.30, 129.65, 128.85, 128.80, 127.05, 126.70, 125.80, 122.05, 121.25, 117.30; MS (ESI): 331 $(M+H)^+$.

[5-(2,3-Dihydro-1-benzofuran-5-yl)-2-thienyl](3-hydroxyphenyl)methanone (**25**). The title compound was prepared by reaction of (5-bromo-2-thienyl)(3-hydroxyphenyl)methanone (**1a**) (150 mg, 0.53 mmol), 2,3-dihydro-1-benzofuran-5-boronic acid (105 mg, 0.64 mmol), caesium carbonate (691 mg, 2.12 mmol) and tetrakis(triphenylphosphine) palladium (6 mg, 5 µmol) according to method A1. The product was purified by CC (hexane/ethyl acetate 7:3); yield: 65 % (111 mg, yellow powder); ^{1}H NMR (CD_3COCD_3): 7.67-7.66 (m, 2H), 7.57-7.55 (m, 1H), 7.44 (d, *J* = 4.1 Hz, 1H), 7.39 (t, *J* = 7.8 Hz, 1H), 7.35 (dt, *J* = 1.3 Hz and *J* = 7.6 Hz, 1H), 7.32-7.31 (m, 1H), 7.11 (ddd, *J* = 1.3 Hz and *J* = 2.5 Hz and *J* = 7.9 Hz, 1H), 6.82 (d, *J* = 8.5 Hz, 1H), 4.63 (t, *J* = 8.8 Hz, 2H), 3.29 (t, *J* = 8.8 Hz, 2H); ^{13}C NMR (CD_3COCD_3): 188.55, 159.35, 141.55, 138.05, 131.50, 130.85, 128.40, 127.80, 125.05, 124.80, 122.00, 121.00, 117.20, 111.45, 73.55, 73.50; MS (ESI): 323 $(M+H)^+$.

[5-(1,3-Benzodioxol-5-yl)-2-thienyl](3-hydroxyphenyl)methanone (**26**). The title compound was prepared by reaction of (5-bromo-2-thienyl)(3-hydroxyphenyl)methanone (**1a**) (150 mg, 0.53 mmol), 1,3-benzodioxole-5-boronic acid (106 mg, 0.64 mmol), caesium carbonate (691

mg, 2.12 mmol) and tetrakis(triphenylphosphine) palladium (6 mg, 5 μmol) according to method A1. The product was purified by CC (hexane/ethyl acetate 8:2); yield: 70 % (120 mg, yellow powder); ^{1}H NMR (CD_3COCD_3): 8.76 (s, 1H), 7.66 (d, J = 4.1 Hz, 1H), 7.46 (d, J = 4.1 Hz, 1H), 7.39 (t, J = 7.8 Hz, 1H), 7.35 (dt, J = 1.3 Hz and J = 7.6 Hz, 1H), 7.33-7.32 (m, 1H), 7.30 (ddd, J = 0.6 Hz and J = 1.9 Hz and J = 8.2 Hz, 1H), 7.28-7.27 (m, 1H), 7.12 (ddd, J = 1.3 Hz and J = 2.5 Hz and J = 7.9 Hz, 1H), 6.93 (d, J = 7.9 Hz, 1H), 6.08 (s, 2H); ^{13}C NMR (CD_3COCD_3): 188.60, 159.40, 154.40, 150.55, 143.35, 141.35, 137.90, 131.55, 129.35, 125.60, 122.35, 122.05, 121.15, 117.25, 110.65, 108.15, 103.70; MS (ESI): 325 $(M+H)^+$.

(3-Hydroxyphenyl)[5-(1*H*-indol-5-yl)-2-thienyl]methanone (**27**). The title compound was prepared by reaction of (5-bromo-2-thienyl)(3-hydroxyphenyl)methanone (**1a**) (150 mg, 0.53 mmol), indol-5-boronic acid (103 mg, 0.64 mmol), caesium carbonate (691 mg, 2.12 mmol) and tetrakis(triphenylphosphine) palladium (6 mg, 5 μmol) according to method A1. The product was purified by CC (hexane/ethyl acetate 7:3); yield: 47 % (80 mg, yellow powder); ^{1}H NMR (CD_3COCD_3): 10.46 (s, 1H), 8.70 (s, 1H), 7.90-7.89 (m, 1H), 7.69 (d, J = 4.1 Hz, 1H), 7.67 (d, J = 8.2 Hz, 1H), 7.53 (d, J = 4.1 Hz, 1H), 7.49 (dd, J = 1.5 Hz and J = 8.2 Hz, 1H), 7.46-7.45 (m, 1H), 7.40 (t, J = 7.6 Hz, 1H), 7.36 (dt, J = 1.5 Hz and J = 7.6 Hz, 1H), 7.34-7.33 (m, 1H), 7.12 (ddd, J = 1.3 Hz and J = 2.5 Hz and J = 7.9 Hz, 1H), 6.54-6.53 (m, 1H); ^{13}C NMR (CD_3COCD_3): 186.40, 159.40, 138.10, 137.50, 131.50, 128.95, 125.05, 122.85, 122.00, 121.00, 119.80, 117.25, 111.10, 103.85; MS (ESI): 320 $(M+H)^+$.

(3-Hydroxyphenyl)[5-(1*H*-indol-6-yl)-2-thienyl]methanone (**28**). The title compound was prepared by reaction of (5-bromo-2-thienyl)(3-hydroxyphenyl)methanone (**1a**) (150 mg, 0.53 mmol), indol-6-boronic acid (103 mg, 0.64 mmol), caesium carbonate (691 mg, 2.12 mmol) and tetrakis(triphenylphosphine) palladium (6 mg, 5 μmol) according to method A1. The product was purified by CC (hexane/ethyl acetate 7:3); yield: 49 % (83 mg, yellow powder); ^{1}H NMR (CD_3COCD_3): 10.48 (s, 1H), 8.77 (s, 1H), 7.90-7.89 (m, 1H), 7.69 (d, J = 4.1 Hz, 1H), 7.66 (d, J = 8.2 Hz, 1H), 7.53 (d, J = 4.1 Hz, 1H), 7.49 (dd, J = 1.5 Hz and J = 8.2 Hz, 1H), 7.45-7.44 (m, 1H), 7.40 (td, J = 0.6 Hz and J = 7.6 Hz, 1H), 7.36 (dt, J = 1.5 Hz and J = 7.6 Hz, 1H), 7.34-7.33 (m, 1H), 7.12 (ddd, J = 1.3 Hz and J = 2.5 Hz and J = 7.9 Hz, 1H), 6.54-6.53 (m, 1H); ^{13}C NMR (CD_3COCD_3): 188.60, 159.40, 156.90, 142.85, 141.60, 138.50, 138.10, 131.50, 131.15, 128.95, 128.45, 125.05, 122.85, 122.00, 121.00, 119.80, 117.25, 111.10, 103.80; MS (ESI): 320 $(M+H)^+$.

(3-Hydroxyphenyl)[5-(2*H*-indazol-5-yl)-2-thienyl]methanone (**29**). The title compound was prepared by reaction of (5-bromo-2-thienyl)(3-hydroxyphenyl)methanone (**1a**) (150 mg, 0.53 mmol), 2*H*-indazole-5-boronic acid (104 mg, 0.64 mmol), caesium carbonate (691 mg, 2.12

mmol) and tetrakis(triphenylphosphine) palladium (6 mg, 5 μmol) according to method A2 refluxing the mixture for 14 h instead of 4 h. The product was purified by CC (hexane/ethyl acetate 6:4); yield: 75 % (127 mg, yellow powder); ^{1}H NMR (CD_3SOCD_3): 13.27 (s, 1H), 9.87 (s, 1H), 8.24 (s, 1H), 8.17 (s, 1H), 7.79 (dd, *J* = 1.5 Hz and *J* = 8.5 Hz, 1H), 7.71 (d, *J* = 4.1 Hz, 1H), 7.66-7.63 (m, 2H), 7.38 (t, *J* = 7.9 Hz, 1H), 7.28 (dt, *J* = 1.3 Hz and *J* = 7.9 Hz, 1H), 7.22-7.21 (m, 1H), 7.06 (ddd, *J* = 0.9 Hz and *J* = 2.5 Hz and *J* = 7.9 Hz, 1H); ^{13}C NMR (CD_3SOCD_3): 186.60, 179.45, 157.35, 153.20, 140.45, 139.85, 138.65, 136.65, 134.35, 129.70, 125.10, 124.65, 124.15, 123.25, 119.50, 118.35, 115.05, 111.05; MS (ESI): 321 $(M+H)^+$.

Biological Methods

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

17β-HSD1 and 17β-HSD2 were obtained from human placenta according to previously described procedures.[52] Fresh human placenta was homogenized and cytosolic fraction and microsomes were separated by 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 17β-HSD1

Inhibitory activities were evaluated by an established method with minor modifications.[52] 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 (1mM). Inhibitor stock solutions were prepared in DMSO. The final concentration of DMSO was adjusted to 1 % in all samples. The enzymatic reaction was started by addition of a mixture of unlabelled- and [2, 4, 6, 7-^{3}H]-E1 (final concentration: 500 nM, 0.15 μCi). After 10 min, the incubation was stopped with $HgCl_2$ and the mixture was extracted with diethylether. After evaporation, the steroids were dissolved in acetonitrile. E1 and E2 were separated using acetonitrile/water (45:55) as mobile phase in a C18 reverse phase chromatography column (Nucleodur C18 Gravity, 3 μm, Macherey-Nagel, Düren) connected to a HPLC-system (Agilent 1100 Series, Agilent Technologies, Waldbronn). Detection and quantification of the steroids were performed using a radioflow detector (Berthold Technologies, Bad Wildbad). The conversion rate was calculated after analysis of the resulting chromatograms according to the following equation: $\%conversion = \frac{\%E2}{\%E2 + \%E1} \times 100$.

Each value was calculated from at least three independent experiments.

Inhibition of 17β-HSD2

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

ER-affinity

The binding affinity of selected compounds to the ERα and ERβ was determined according to Zimmermann et al.[58] Briefly, 0.25 pmol of ERα or ERβ, respectively, were incubated with [2, 4, 6, 7-^{3}H]-E2 (10 nM) and test compound for 1 h at room temperature. The potential inhibitors were dissolved in DMSO (5 % final concentration). Evaluation of non-specific-binding was performed with diethylstilbestrol (10 µM). After incubation, ligand-receptor complexes were selectively bound to hydroxyapatite (5 g/ 60 mL TE-buffer). The complex formed was separated, washed and resuspended in ethanol. For radiodetection, scintillator cocktail (Quickszint 212, Zinsser Analytic, Frankfurt) was added and samples were measured in a liquid scintillation counter (Rack Beta Primo 1209, Wallac, Turku). For determination of the relative binding affinity (RBA), inhibitor and E2 concentrations required to displace 50 % of the receptor bound labeled E2 were determined. RBA values were calculated according to the following equation: $RBA[\%] = \frac{IC_{50}(E2)}{IC_{50}(compound)} \times 100$.

The RBA value for E2 was set at 100 %.

Inhibition of 17β-HSD1 in T47D cells

A stock culture of T47D cells was grown in RPMI 1640 medium supplemented with 10 % FCS, L-glutamine (2 mM), penicillin (100 IU/mL), streptomycin (100 µg/mL), insulin-zinc-salt (10 µg/mL) and sodium pyruvate (1 mM) at 37 °C under 5 % CO_2 humidified atmosphere. The cells were seeded into a 24-well plate at 1x10^6 cells/well in DMEM medium with FCS, L-glutamine and the antibiotics added in the same concentrations as mentioned above. After 24 h the medium was changed for fresh serum free DMEM and a solution of test compound in DMSO was added. Final concentration of DMSO was adjusted to 1 % in all samples. After a pre-incubation of 30 min at 37 °C with 5 % CO_2, the incubation was started by addition of a mixture of unlabelled- and [2, 4, 6, 7-^{3}H]- E1 (final concentration: 50 nM, 0.15 µCi). After 0.5 h incubation, the enzymatic reaction was stopped by removing of the supernatant medium. The steroids were extracted with diethylether. Further treatment of the samples was carried out as mentioned for the 17β-HSD1 assay.

Metabolic stability

Human liver microsomes (final protein concentration 0.5 mg/mL), 0.1 M phosphate buffer pH 7.4 and test compound (final substrate concentration = 3 µM; final DMSO concentration = 0.25 %) were preincubated at 37 °C prior to the addition of NADPH (final concentration = 1 mM) to initiate the reaction. Dextromethorphan and verapamil were used as references. All incubations were performed singularly for each test compound. Each compound was incubated for 0, 5, 15, 30 and 45 min (control: 45 min). The reactions were stopped by the addition of 50 µL methanol containing internal standard at the appropriate time points. The incubation plates were centrifuged at 2,000 rpm for 20 min at 4 °C to precipitate the protein. The sample supernatants were combined in cassettes of up to 4 compounds and analyzed using LC-MS/MS.

Molecular Modeling

Docking studies were performed using the AutoDock4.2[59] program. Atomic coordinates of 17β-HSD1 were derived from the structure of the ternary complex 17β-HSD1, $NADP^+$ and E2 (PDB-ID: 1FDT (conformation B for residues 187-200)). The crystal structure was energy minimized with MOE2009.10 (Chemical Computing Group Inc., Montreal, Canada) applying the AMBER99 force field and generalized Born model, keeping the coordinates of backbone atoms fixed. Ligand structures were built, assigned AM1-BCC charges, and minimized in MOE with the MMFF94x force field. The 17β-HSD1 x-ray structure and ligand structures were prepared for docking studies through the graphical user interface AutoDockTools1.5.4.[59] For the ligands, non-polar hydrogen atoms were deleted, rotatable bonds were defined and AM1-BCC charges kept. For the protein, non-polar hydrogen atoms were deleted and charges were added to the structure. The docking area has been defined by a box, centered on the steroid binding site. Grid points of 90 x 90 x 90 with 0.250 Å spacing were calculated around the docking area for all the ligand atom types using AutoGrid4.2. For each inhibitor, 50 separate docking calculations were performed. Each docking calculation consisted of $25x10^5$ energy evaluations using the Lamarckian genetic algorithm local search (GALS) method. Each docking run was performed with a population size of 250. A mutation rate of 0.02 and a crossover rate of 0.8 were used to generate new docking trials for subsequent generations. The docking results from each of the 50 calculations were clustered on the basis of root-mean-square deviation (RMSD = 2.0 Å) between the Cartesian coordinates of the ligand atoms and were ranked on the basis of the free binding energy.

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4 DISKUSSION

Das Ziel dieser Arbeit war die Entwicklung neuer Hemmstoffe der 17β-HSD1, die potentielle Wirkstoffe zur Behandlung estrogenabhängiger Krankheiten darstellen. Eine Kombination aus struktur- und ligandbasiertem Designansatz wurde genutzt, um neue Moleküle zu entwickeln, die sowohl die 17β-HSD1 hemmen als auch selektiv gegenüber der 17β-HSD2 (katalysiert die Umkehrreaktion: Oxidation von E2 zu E1) sowie gegenüber den Estrogenrezeptoren α und β (um jegliche intrinsische estrogene Aktivität zu vermeiden) sind.
Die vielversprechendsten Substanzen sollen nicht nur potentielle Wirkstoffkandidaten darstellen, sondern auch für die Erbringung eines *in vivo* proof of concepts mittels nichtsteroidaler Inhibitoren in Frage kommen.

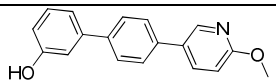
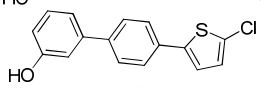
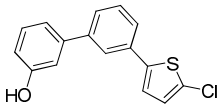
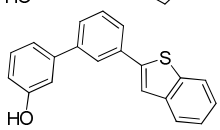
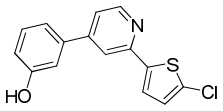
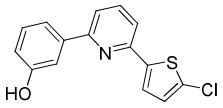
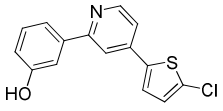
Kapitel 3.1 handelt von dem Design, der Synthese und der biologischen Evaluierung von 20 Verbindungen, die als Substanzklasse der heterocyclisch substituierten Biphenylole bezeichnet wird. Der Ausgangspunkt des Designkonzeptes ist das natürliche Substrat Estron, welches mit seinen strukturellen Merkmalen als Templat dient. Nachdem sich ein *meta*-Hydroxyphenylring innerhalb anderer arbeitskreisinternen Verbindungsklassen (Hydroxyphenylnaphthole und Bis(hydroxyphenyl)heterocyclen) als guter Ersatz für den steroidalen A-Ring und als wichtiges Strukturmerkmal für Hemmaktivität herausstellte, wurde diese Teilstruktur beibehalten. Ein Schwerpunkt wurde auf das Imitieren des steroidalen D-Ringes gelegt. Im Gegensatz zu den vorangegangenen Substanzklassen wurde auf eine weitere Hydroxygruppe verzichtet und versucht, die Funktion der Ketogruppe durch einen Heterocyclus bzw. einen reinen Wasserstoffbrückenakzeptor zu ersetzen. Verbunden wurden die jeweiligen Teilstrukturen durch einen Phenylring, der den hydrophoben Kern des Steroids nachahmen soll. Im Verlauf der Studie wurde der mittlere Benzolring gegen Pyridin ausgetauscht, um drei hydrophilen Aminosäuren (Asn152, Tyr218, Ser222) in der überwiegend hydrophoben Substratbindetasche einen weiteren potentiellen Wechselwirkungspartner anzubieten.

Aus dem Pool der unterschiedlichen Heterocyclen, die nach dem Designkonzept mit dem katalytischen Zentrum wechselwirken sollten, konnte nur durch den Einbau aromatischer Ringsysteme Hemmaktivität erzielt werden. Weiterhin wurden folgende Faktoren entdeckt, die innerhalb dieser Substanzklasse hinsichtlich der 17β-HSD1-Hemmung wichtige Rollen spielen:

- Substitution der D-Ring imitierenden Heterocyclen: Vergleiche Verbindungen **I.12**‡ (Chlor) und **I.14** (Methyl)
- Substitutionsmuster des mittleren Ringes: Vergleiche Verbindungen **I.10** (1,4-disubstituiert) und **I.12** (1,3-disubstituiert)
- Position des Pyridin-Stickstoffs bei Verbindungen mit Pyridin als mittlerem Ring: Verbindungen **I.17**-**I.20**

Die Verbindungen, die innerhalb dieser Studie die besten biologischen Profile hinsichtlich Aktivität und Selektivität aufwiesen, sind in Tabelle 1 aufgeführt.

Tabelle 1: *Hemmung der 17β-HSD1, 17β-HSD2 und Bindungsaffinitäten für die Estrogenrezeptoren α und β ausgesuchter Verbindungen*

Verb.	Struktur	IC_{50}[a] (µM)		RBA[d] (%)	
		17β-HSD1[b]	17β-HSD2[c]	ERα	ERβ
I.5		0,69	2,97	0,001<x<0,01	<0,001
I.10		1,02	>10	0,01<x<0,1	0,001<x<0,01
I.12		0,56	2,37	<0,01	<0,001
I.15		1,37	1,94	<0,01	<0,001
I.17		2,38	0,47	0,001<x<0,01	<0,001
I.18		1,39	7,11	0,001<x<0,01	<0,001
I.19		0,85	3,64	<0,001	<0,001

[a] Mittelwert aus drei Messungen, Standardabweichung unter 15 %
[b] Humane Plazenta, cytosolische Fraktion, Substrat [^{3}H]-E1, 500 nM, Cofaktor NADH, 500 µM
[c] Humane Plazenta, microsomale Fraktion, Substrat [^{3}H]-E2, 500 nM, Cofaktor NAD^+, 1500 µM
[d] RBA: relative Bindungsaffinät, E2: 100 %, Mittelwert aus drei Messungen, Standardabweichung unter 10 %

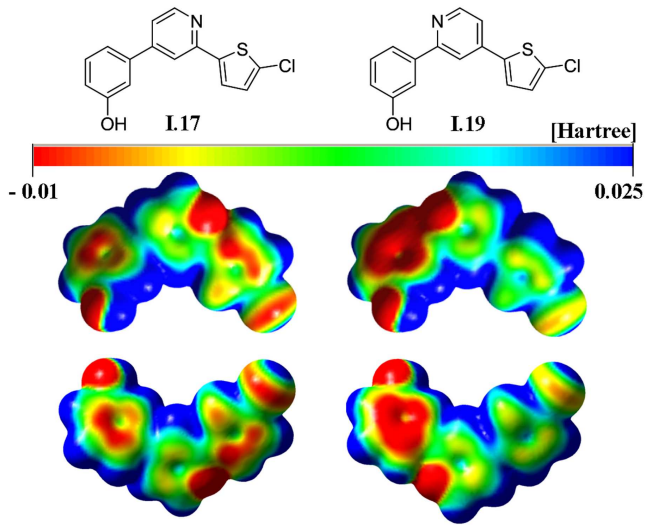
Der Austausch des mittleren Benzolringes gegen ein Pyridin bzw. die Änderung der Position des Pyridinstickstoffs, führte zu Variationen hinsichtlich Aktivität und Selektivität, wobei jedoch kein Derivat die geplante Aktivitätssteigerung (durch zusätzliche

‡ Alle in Kapitel 4 erwähnten Verbindungen wurden sowohl mit den römischen Nummern **I**-**III** charakterisiert, die die jeweilige Publikation repräsentieren, in der die Verbindung beschrieben wird, als auch mit einer arabischen Nummer, die für die entsprechende Nummer in der Publikation steht. (z.B. **I.12** ist Verbindung **12** aus Publikation **I**)

Wasserstoffbrückenbindungen) hervorrufen konnte. Die unterschiedlichen biologischen Daten der Pyridin-Derivate könnten vielleicht auf die veränderten Molekularen Elektrostatischen Potentialverteilungen (MEP) zurückzuführen sein, deren wichtige Rolle bei der Hemmung der 17β-HSD1 bereits beschrieben wurde [Bey 2008b, 2009].

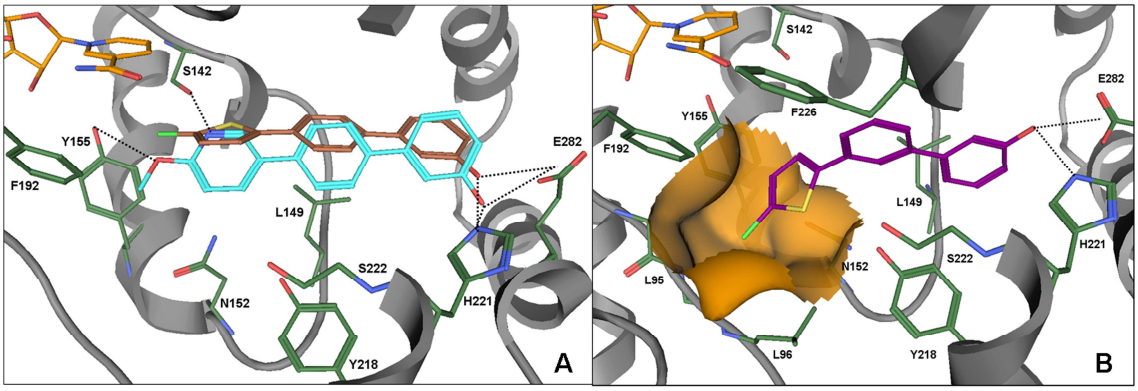
Vor allem hinsichtlich der Selektivität gegenüber der 17β-HSD2 sind die Verbindungen **I.17** bis **I.20** stark abhängig von der Lage des Pyridinstickstoffs. Besonders markant sind die Auswirkungen bei **I.17** (Stickstoff befindet sich neben Thiophen) und **I.19** (Stickstoff befindet sich neben Hydroxyphenyl). Hier wird abhängig vom Pyridinstickstoff die Selektivität vollständig umgewandelt und ein Hemmstoff der 17β-HSD1 (**I.19**) zu einem 17β-HSD2 Hemmstoff (**I.17**). Da bis heute weder eine Kristallstruktur noch ein geeignetes Homologiemodell der 17β-HSD2 verfügbar ist, können Protein-Ligand-Wechselwirkungen nicht interpretiert werden. Wenn man in diesem Zusammenhang jedoch die MEPs der beiden Verbindungen berechnet und vergleicht, erkennt man (Abbildung 12), dass die Anwesenheit des Stickstoffs zu einem negativeren elektrostatischen Potential in dem jeweiligen Bereich des Moleküls führt. Die elektrostatischen Verteilungen der Verbindungen könnten sich entscheidend auf die Affinität zu den jeweiligen Enzymen auswirken und somit für die höhere bzw. niedrigere Hemmaktivität verantwortlich sein.

Abbildung 12: *Struktur der Verbindungen **I.17** und **I.19** sowie deren MEP-Berechnungen in Vorder- und Rückansicht*



Um genauere Einblicke in die Protein-Ligand-Wechselwirkungen der potentesten Substanzen dieser Studie zu bekommen, wurde die Bindung ausgewählter Verbindungen in einer Molekularen Docking Studie untersucht. Die wahrscheinlichsten Bindungsposen der Verbindungen **I.5**, **I.10** und **I.12** sind in Abbildung 11 dargestellt.

*Abbildung 13: Gedockter Komplex zwischen 17β-HSD1 (PDB-ID: 1EQU) und der Überlagerung der Verbindungen **I.5** (cyan) und **I.10** (braun) ist dargestellt in Abbildung 13A, zwischen 17β-HSD1 und **I.12** (violett) in Abbildung 13B.*



Abgebildet sind der Cofaktor NADPH (orange), an Wechselwirkungen beteiligte Reste (grün) und die in der Darstellungsweise Cartoon wiedergegebene Tertiärstruktur (grau) der Substratbindestelle der 17β-HSD1. Wasserstoffbrückenbindungen sind in schwarz-gestrichelten Linen angegeben. Die Oberfläche der neuen zusätzlichen Bindetasche ist in Abbildung 13B dargestellt (hellbraun). Die Bilder wurden generiert mit MOE (Chemical Computing Group Inc., Montreal, Kanada).

Die Verbindungen **I.5** und **I.10** imitieren erwartungsgemäß das Bindungsverhalten des natürlichen steroidalen Substrates. Während der Phenolring mit His221 und Glu282 Wasserstoffbrückenbindungen eingeht, befindet sich der Heterocyclus im katalytischen Zentrum.

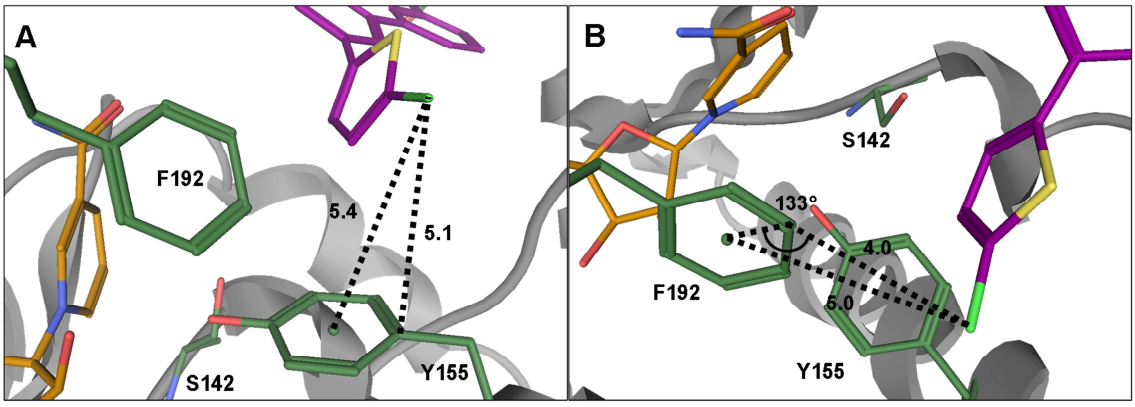
Obwohl eine Wasserstoffbrückenbindung zwischen Ser142 und Tyr155 postuliert wurde [Ghosh 2001] und somit eine der beiden Hydroxygruppen der Aminosäureseitenketten als Wasserstoffbrückendonor nicht mehr zur Verfügung stehen würde, zeigen die Ergebnisse in Abbildung 13A, dass Verbindung **I.5** zwei Wasserstoffbrückenbindungen mit den beiden Aminosäuren eingeht. Der Methoxysauerstoff fungiert dabei als Akzeptor für das Tyr155-OH und der Pyridinstickstoff für das Ser142-OH. Unklar ist noch, ob die Interaktionen, welche in Abbildung 13A dargestellt sind, für die Stabilisierung von **I.5** relevant sind, da die ähnlich bindende Chlorthiophen Teilstruktur des äquipotenten Hemmstoffs **I.10** solche Wasserstoffbrücken-Wechselwirkungen nicht eingehen kann. Die exakte Rolle des Chlorthiophens dieser linearen Verbindung **I.10** müsste daher noch genauer untersucht werden.

Interessanterweise fand man die gewinkelten Hemmstoffe **I.12**–**I.15** in einem abweichenden Bindungsmodus. Obwohl diese Verbindungen, gemäß dem Designkonzept, mit den anderen beiden Verbindungen (**I.5** und **I.10**) überlagern sollten, deuten die Ergebnisse darauf hin, dass zwar deren Hydroxyphenyl-Part mit denen der beiden linearen Substanzen überlagert, der heterocyclische Teil aber anders bindet. Dieser ragt in eine überwiegend hydrophobe zusätzliche Bindetasche unter dem katalytischen Zentrum. Diese Tasche wird überwiegend geformt aus den Aminosäuren Leu95, Leu96, Asn152, Tyr155 und Phe192 und wurde bereits als Wechselwirkungsort für den steroidalen Hemmstoff E2B beschrieben [Mazumdar 2009]. In

Abbildung 13B ist die Docking Pose des aktivsten Hemmstoffes dieser Studie (**I.12**) inklusive der hellbraun gefärbten Oberfläche der zusätzlichen Bindungstasche abgebildet. Innerhalb dieser lipophilen Bindetasche könnten hydrophobe und van der Waals-Wechselwirkungen für die Affinität der Verbindungen ausschlaggebend sein. Die biologischen Daten der Verbindungen **I.13**-**I.15** bestätigen den Bindungsmodus der gewinkelten Hemmstoffe, da z.B. der Benzothiophensubstituent von **I.15** aufgrund seiner sterischen Ausmaße bei dem erwarteten E2-ähnlichen Bindungsmodus nicht in das katalytische Zentrum passen würde.
Verglichen mit **I.12** (IC_{50} = 0,56 µM), stellt sich die unsubstituierte Verbindung **I.13** (48 % Hemmung bei Inhibitorkonzentration von 1 µM) als schwächer aktive Verbindung heraus, was auf die geringere Lipophilie des Wasserstoffs im Vergleich zu Chlor zurückzuführen sein könnte. Diese These wird jedoch wieder in Frage gestellt, weil auch Verbindung **I.14** mit Methylgruppe, welche eine nahezu gleiche Lipophilie wie Chlor aufweist, eine schlechtere Hemmaktivität (32 % Hemmung bei Inhibitorkonzentration von 1 µM) hervorruft. In diesem Zusammenhang müssten weiterhin sowohl die unterschiedlichen elektronischen Charaktere der Substituenten als auch das mögliche Auftreten von Cl-π-Interaktionen [Matter 2009] untersucht werden.

Um die Möglichkeit der Cl-π Interaktionen von **I.12** mit der neuen zusätzlichen Bindetasche zu erforschen, muss man zuerst die räumliche Anordnung zwischen dem Chlorsubstituent und den π-Systemen der aromatischen Aminosäuren Tyr155, Phe192 und Phe226, die alle in diesem lipophilen Areal liegen, betrachten. Abbildung 14 zeigt vergrößerte Abbildungen der Docking Pose von **I.12** mit besonderem Fokus auf potentiellen Cl-π Interaktionen.

*Abbildung 14: Detaillierte Ansicht potentieller Cl-π Interaktionen von **I.12** (violett) innerhalb der zusätzlichen Bindungstasche (Kristallstruktur PDB-ID: 1EQU)*

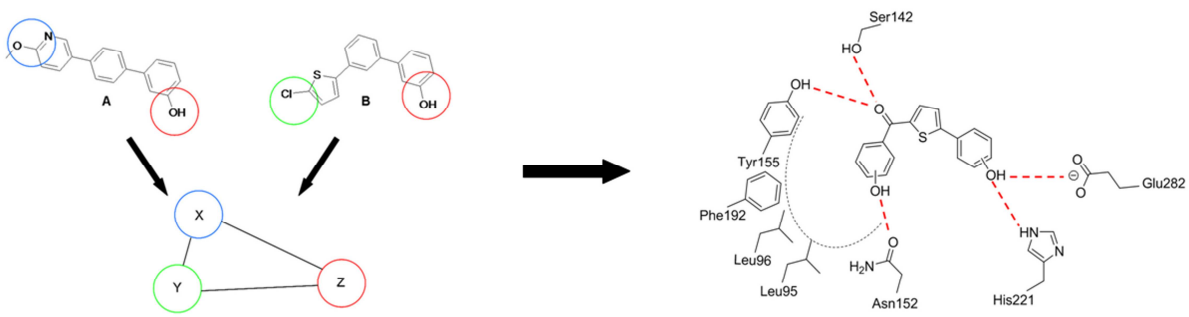


Abgebildet sind der Cofaktor NADPH (orange), an Wechselwirkungen beteiligte Reste (grün) und die in der Darstellungsweise Cartoon wiedergegebene Tertiärstruktur (grau) der Substratbindestelle der 17β-HSD1. Abstände *d* der Cl-π Interaktionen sind in schwarz-gestrichelten Linien und in der Maßeinheit Å angegeben. Abbildung 14A zeigt "face-on" Geometrie; Abbildung 14B "edge-on". Die Bilder wurden generiert mit MOE (Chemical Computing Group Inc., Montreal, Canada).

Das Chloratom ist 5,4 Å vom Zentroid des aromatischen Tyr155-Ringsystems und 4,0 Å vom nächstgelegenen aromatischen Kohlenstoff des Phe192 entfernt, was Cl-π Interaktionen ermöglicht. In Abhängigkeit von der geometrischen Orientierung des Chlors relativ zum aromatischen Ring kann man innerhalb der Cl-π Interaktionen unterscheiden zwischen „edge-on“ und „face-on“ Wechselwirkungen [Imai 2008, 2009]. Die Interaktion von **I.12** mit Tyr155 ($d_{\text{Cl-Zentroid}}$ = 5.4 Å und $d_{\text{Cl-C}}$ = 5.1 Å) zeigt „face-on“ Geometrie (Abbildung 14A). Tyrosin ist bekanntermaßen ein geeigneter Interaktionspartner für „face-on“ Wechselwirkungen, während Phenylalanin eher die „edge-on“ Geometrie bevorzugt [Imai 2008, Lu 2007]. Die relative Orientierung von Phe192 zu Verbindung **I.12** ($d_{\text{Cl-Zentroid}}$ = 5.0 Å und $d_{\text{Cl-C}}$ = 4.0 Å) deutet auf dieses Wechselwirkungsmuster hin (Abbildung 14B). Im Prinzip liegt hier auch eine passende Orientierung zur Ausbildung von CH-Cl Interaktionen vor [Imai 2008], wobei der Winkel (θ = 133°) des Chloratoms relativ zur aromatischen Ringebene von Phe192 (Abbildung 14B) eher auf Cl-π Interaktionen („edge-on“) schließen lässt.
Aufgrund der Molecular Modelling Ergebnisse könnte also die erhöhte Hemmaktivität des Chlorthiophen-Derivates (**I.12**) gegenüber **I.13-I.15** auf Cl-π Wechselwirkungen des Liganden mit den Aminosäuren Tyr155 und Phe192 zurückzuführen sein.

In Kapitel 3.2 wurde das Targeting der zusätzlichen Bindetasche unterhalb des katalytischen Zentrums aufgegriffen und in ein neues Pharmakophormodell integriert. Aus den in Abbildung 13 gezeigten Bindungsmodi der Inhibitoren **I.5** und **I.12** wurde ein 3-Punkt-Pharmakophormodell entwickelt, welches den Ausgangspunkt für das Design einer neuen Substanzklasse von 17β-HSD1 Hemmstoffen, den bicyclisch substituierten Hydroxyphenylmethanonen, darstellt (Abbildung 15).

Abbildung 15: *3-Punkt-Pharmakophormodell und daraus resultierende schematische Präsentation der designten Leitstruktur und ihrer potentiellen Interaktionen mit den Aminosäuren der Substratbindestelle der 17β-HSD1 (mögliche Wasserstoffbrückenbindungen sind mit rot-gestrichelten Linien gekennzeichnet)*



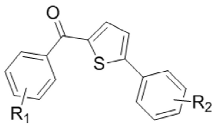
X = reiner Wasserstoffbrückenakzeptor (Keton): WW mit Ser142 und Tyr155 (katalytisches Zentrum)

Y = Wasserstoffbrückendonor + lipophiler Part (Phenol): WW mit Asn152 in lipophiler zusätzlicher Bindetasche

Z = Wasserstoffbrückendonor und/oder –akzeptor (OH): WW mit His221 und Glu282 in C-terminaler Region

Als reiner Wasserstoffbrückenakzeptor, der im katalytischen Zentrum interagieren soll, wird eine Ketofunktion verwendet. Diese funktionelle Gruppe kann nicht nur die erwarteten Interaktionen ausbilden, sie verschafft dem gesamten Molekül mit ihrem sp2-hybridisierten Kohlenstoff auch einen Winkel, der es erlaubt, den Punkt Y des Pharmakophormodells (überwiegend lipophile Bindetasche) zu erreichen, die sich unterhalb des katalytischen Zentrums befindet. Bei genaueren Untersuchungen dieser dritten Region, wurde die Aminosäure Asn152 als einzige hydrophile Aminosäure innerhalb dieser Tasche ausgemacht. Weiterhin wurde ein hydrophober Kern (Thiophen) zur Verbindung der genannten Teilstücke gewählt, um sowohl die hydrophobe Substratbindestelle (bekannt als hydrophober Tunnel [Bey 2008a]) auszufüllen als auch um die Teilstrukturen optimal auszurichten (Abbildung 15).
Die biologischen Ergebnisse belegen den Erfolg dieses Designkonzeptes. Die erste Verbindung dieser Studie **II.1** (Derivat der o.g. Leitstruktur mit *meta-meta*-OH-Substitutionsmuster) zeigt einen IC_{50}-Wert von 22 nM (Tabelle 2). Interessanterweise hat die Position der Hydroxygruppe an dem Phenylring, der direkt mit dem Thiophen verbunden ist, geringeren Einfluss auf die Hemmaktivität als die OH-Funktion des anderen Phenylringes. Entweder sind die vermuteten Wasserstoffbrückenbindungen zu His221 und Glu282 weniger wichtig für eine hohe Hemmaktivität oder das Protein ist in dieser C-terminalen Region so flexibel, dass die Hydroxygruppe auch in den unterschiedlichen Positionen von **II.1**, **II.4** und **II.5** die gleichen Interaktionen ausüben kann. Die Rolle der OH-Funktion des Thiophen-verknüpften Phenols wird in Kapitel 3.3 weiter beleuchtet.

Tabelle 2: *Hemmung der 17β-HSD1 und 17β-HSD2 durch Leitstrukturen*



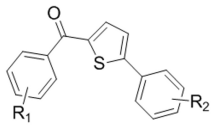
Verb.	R_1	R_2	IC_{50} [nM][a]		SF[d]
			17β-HSD1[b]	17β-HSD2[c]	
II.1	*m*-OH	*m*-OH	22	109	5
II.2	*p*-OH	*m*-OH	368	376	1
II.3	*o*-OH	*m*-OH	945	567	0,6
II.4	*m*-OH	*p*-OH	33	478	15
II.5	*m*-OH	*o*-OH	95	18	0,2

[a] Mittelwert aus drei Messungen, Standardabweichung unter 17 %
[b] Humane Plazenta, cytosolische Fraktion, Substrat [^{3}H]-E1, 500 nM, Cofaktor NADH, 500 µM
[c] Humane Plazenta, microsomale Fraktion, Substrat [^{3}H]-E2, 500 nM, Cofaktor NAD^+, 1500 µM
[d] SF = Selektivitätsfaktor = IC_{50} (17β-HSD2) / IC_{50} (17β-HSD1)

Innerhalb der lipophilen Bindetasche unter dem katalytischen Zentrum sind zu den Positionen der OH-Funktionen auch durch das Einführen von zusätzlichen Substituenten scharfe Struktur-Wirkungsbeziehungen auszumachen (Tabelle 3). Während Methyl-Substituenten in *para*-

Position (Verbindungen **II.6** und **II.8**) zu einem starken Aktivitätsverlust führen, erreichen Verbindungen mit Fluor-Substituenten an der gleichen Position (**II.7** und **II.9**) gute Hemmungen der 17β-HSD1. Offenbar haben in dieser Region sowohl der sterische Anspruch eines Substituenten als auch dessen elektronische Fähigkeiten Einfluss auf die inhibitorische Potenz. Folgerichtig liegt der 17β-HSD1 Hemmwert von Verbindung **II.10** mit Nitro-Substituent, welcher sterisch sehr groß ist (noch größer als Methyl) und elektronenziehende Eigenschaften hat (wie Fluor), zwischen denen der Methyl- und Fluor-Verbindungen.

Tabelle 3: *Hemmung der 17β-HSD1 und 17β-HSD2 durch Verbindungen mit zusätzlichen Substituenten am Benzoyl*

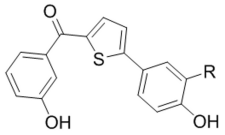


Verb.	R_1	R_2	IC_{50} [nM][a]		SF[d]
			17β-HSD1[b]	17β-HSD2[c]	
II.6	CH_3	*p*-OH	52[e]	13[e]	
II.7	F	*p*-OH	21	69	3
II.8	CH_3	*m*-OH	64[e]	31[e]	
II.9	F	*m*-OH	18	49	3
II.10	NO_2	*m*-OH	594	240	0,4

[a] Mittelwert aus drei Messungen, Standardabweichung unter 18 %
[b] Humane Plazenta, cytosolische Fraktion, Substrat [^{3}H]-E1, 500 nM, Cofaktor NADH, 500 µM
[c] Humane Plazenta, microsomale Fraktion, Substrat [^{3}H]-E2, 500 nM, Cofaktor NAD^+, 1500 µM
[d] SF = Selektivitätsfaktor = IC_{50} (17β-HSD2) / IC_{50} (17β-HSD1)
[e] % Hemmung bei Inhibitorkonzentration von 1 µM

Kleine Substituenten wurden am Phenol vor allem in *ortho*-Position zur *para*-Hydroxygruppe eingefügt. Bei näherer Betrachtung fällt auf, dass Verbindungen mit lipophilen Substituenten wie Methyl (**II.14**), Fluor (**II.15**), Chlor (**II.17**) und Ethyl (**II.20**) hervorragende Hemmaktivitäten (IC_{50}-Werte im niedrigen z.T. einstelligen nanomolaren Bereich) und Selektivität gegenüber der 17β-HSD2 (Selektivitätsfaktoren zwischen 30 und 50) aufweisen (Tabelle 4). Um einen Einblick in die Protein-Ligand-Wechselwirkungen bezüglich dieser substituierten Position zu bekommen, wurden Molekulare Docking Studien durchgeführt. Repräsentativ für die vier Hemmstoffe ist in Abbildung 17 der gedockte Komplex des Methyl-Derivates **II.14** mit der 17β-HSD1 dargestellt.

Tabelle 4: *Hemmung der 17β-HSD1 und 17β-HSD2 durch Verbindungen mit zusätzlichen Substituenten in ortho-Position zur para-OH*

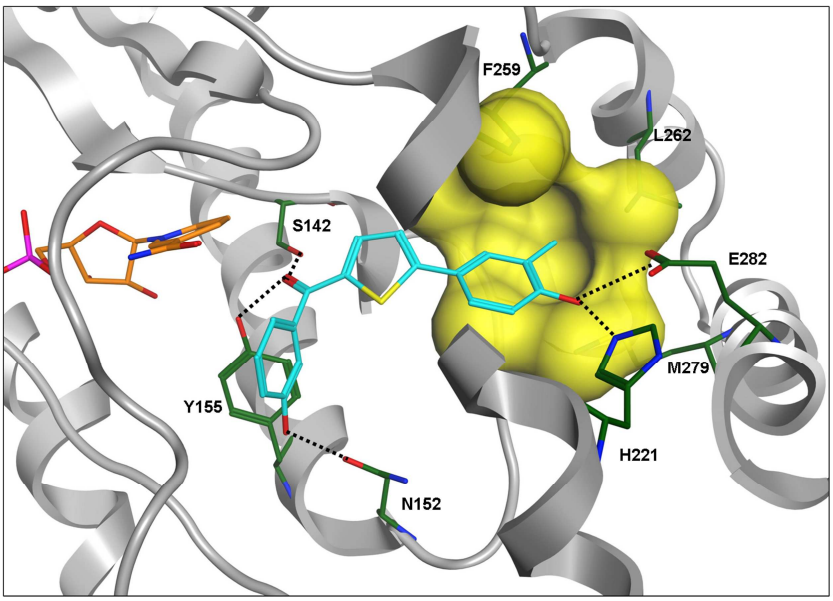


Verb.	R	IC_{50} [nM][a]		SF[d]
		17β-HSD1[b]	17β-HSD2[c]	
II.14	CH_3	8	382	48
II.15	F	19	588	31
II.16	CF_3	14	95	7
II.17	Cl	5	246	49
II.18	OH	86	590	7
II.19	OCH_3	108	793	7
II.20	CH_2CH_3	20	786	39

[a] Mittelwert aus drei Messungen, Standardabweichung unter 23 %
[b] Humane Plazenta, cytosolische Fraktion, Substrat [^{3}H]-E1, 500 nM, Cofaktor NADH, 500 µM
[c] Humane Plazenta, microsomale Fraktion, Substrat [^{3}H]-E2, 500 nM, Cofaktor NAD^+, 1500 µM
[d] SF = Selektivitätsfaktor = IC_{50} (17β-HSD2) / IC_{50} (17β-HSD1)

Innerhalb dieser Region, welche die untersuchte Position (*ortho*-Position zur *para*-OH) umgibt, befinden sich überwiegend lipophile Aminosäuren (Val143, Met147, Phe159, Leu262, Tyr275, Met279), die eine kleine hydrophobe Tasche bilden, welche von den eingeführten Substituenten ausgefüllt wird (Abbildung 16). Verbindungen mit polareren Substituenten wie OH (**II.18**) und Methoxy (**II.19**) zeigen daher geringere Hemmaktivitäten. Da das voluminösere Ethyl-Derivat **II.20** trotz des etwas schlechteren Selektivitätsfaktors (verglichen mit **II.14**) eine geringere Aktivität an der 17β-HSD2 zeigt, könnte eine weitere Vergrößerung des Substituenten (z.B. Propyl, Isopropyl, usw.) in dieser Position zu einer zusätzlichen Selektivitätssteigerung gegenüber dem biologischen Gegenspieler führen. Nach diesen Ergebnissen könnte man spekulieren, ob bei der 17β-HSD2 diese kleine Bindetasche überhaupt vorhanden ist bzw. ob diese vielleicht aus eher hydrophilen Aminosäuren gebildet wird. Es wäre zu überprüfen, ob größere lipophile Substituenten diese kleine Bindetasche der 17β-HSD1 noch besser ausfüllen könnten, wobei man anhand der Docking-Ergebnisse auch mutmaßen kann, dass ein Phenyl-Substituent in dieser Position sterisch womöglich schon zu anspruchsvoll wäre und den Liganden in eine ungünstigere Position zwingen würde.

*Abbildung 16: Gedockter Komplex zwischen 17β-HSD1 (PDB-ID: 1FDT-B) und **II.14** (cyan)*

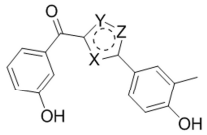


Abgebildet sind der Cofaktor NADPH (orange), an Wechselwirkungen beteiligte Reste (grün) und die in der Darstellungsweise Cartoon wiedergegebene Tertiärstruktur (grau) der Substratbindestelle der 17β-HSD1. Wasserstoffbrückenbindungen sind in schwarz-gestrichelten Linen angegeben. Die Oberfläche der Aminosäuren, die die kleine, den Methylsubstituenten umgebende, hydrophobe Tasche bilden, ist in gelb dargestellt. Das Bild wurde generiert mit MOE (Chemical Computing Group Inc., Montreal, Kanada).

Wie in der strukturell verwandten 17β-HSD1-Hemmstoffklasse der Bis(hydroxyphenyl)heterocyclen [Al-Soud 2009, Bey 2008a, 2008b, 2009] erkennt man auch in der hier beschriebenen Substanzklasse der bicyclisch substituierten Hydroxyphenylmethanone, dass der mittlere Heterocyclus eine entscheidende Rolle für das inhibitorische Profil eines Moleküls spielt. Der Austausch des 2,5-disubstituierten Thiophens (**II.14**) mit dem 2,4-disubstituierten Thiophen (**II.21**) oder unterschiedlichen Thiazolen (**II.22-II.24**) offenbart den wichtigen Einfluss der Position des Schwefelatoms innerhalb des Ringes auf die Enzymhemmung (Tabelle 5). Nur Verbindungen, bei denen der Schwefel zwischen den beiden Substituenten lokalisiert ist, zeigen Hemmwerte im niedrigen nanomolaren Bereich. Nachdem jedoch bereits gezeigt wurde, dass Inhibitoren mit 2,4-disubstituiertem Thiophen die 17β-HSD1 stark hemmen können [Bey 2008b], könnte man als möglichen Grund für die hier erhaltenen unterschiedlichen IC_{50}-Werte wiederum die Molekularen Elektrostatischen Potentiale in Betracht ziehen.

Bei den Thiazol-Verbindungen ist sogar ein starker Abfall der inhibitorischen Potenz zu verzeichnen, sobald das Stickstoffatom zwischen den beiden Substituenten liegt. Interessanterweise hemmen diese beiden Substanzen (**II.23** und **II.24**) die 17β-HSD2 stärker als den Typ 1. Während Verbindung **II.22** sogar ein vergleichbar gutes inhibitorisches Profil wie das 2,5-disubstituierte Thiophen-Analogon **II.14** hat, führt also eine einfache Änderung des Substitutionsmusters am mittleren Heteroaromaten zu einer Umwandlung von einem 17β-HSD1-Hemmstoff zu einem 17β-HSD2-Inhibitor.

Tabelle 5: *Hemmung der 17β-HSD1 und 17β-HSD2 durch Derivate der **II.14** mit variierendem mittleren Heterocyclus*



Verb.	X	Y	Z	IC_{50} [nM][a]		SF[d]
				17β-HSD1[b]	17β-HSD2[c]	
II.21	CH	CH	S	199	719	4
II.22	S	CH	N	35	485	14
II.23	N	S	CH	32[e]	51[e]	
II.24	N	CH	S	19[e]	57[e]	

[a] Mittelwert aus drei Messungen, Standardabweichung unter 13 %
[b] Humane Plazenta, cytosolische Fraktion, Substrat [^{3}H]-E1, 500 nM, Cofaktor NADH, 500 µM
[c] Humane Plazenta, microsomale Fraktion, Substrat [^{3}H]-E2, 500 nM, Cofaktor NAD^+, 1500 µM
[d] SF = Selektivitätsfaktor = IC_{50} (17β-HSD2) / IC_{50} (17β-HSD1)
[e] % Hemmung bei Inhibitorkonzentration von 1 µM

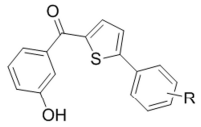
Aufgrund des bislang besten inhibitorischen Profils und der chemisch guten Zugänglichkeit wurde das 2,5-disubstituierte Thiophen für weitere Studien, die in Kapitel 3.3 beschrieben sind, beibehalten. Hier wurde der Hauptfokus auf den Thiophen-verknüpften Ring gerichtet, bei dem die Relevanz der Hydroxygruppe untersucht wurde. Wie bereits in Kapitel 3.2 festgestellt wurde, führte die Positionsänderung der OH-Funktion an diesem Ring zu keinem nennenswerten Aktivitätsgewinn/-verlust. Die These, dass diese Hydroxygruppe keine entscheidende Rolle für die 17β-HSD1 Hemmung spielt, wurde unterstrichen durch die Tatsache, dass weder das Phenyl-Derivat (**III.1**) noch die Methoxy-Verbindungen (**III.2**-**III.4**) einen starken Abfall der Hemmaktivität zeigten (Tabelle 6). Im Gegensatz zum ursprünglichen Designkonzept (Kapitel 3.2), in dem diese OH-Funktion als Wechselwirkungspartner (Wasserstoffbrückenbindungen) mit His221 und Glu282 in der C-terminalen Region der Substratbindetasche vorgesehen war, stellt sich hier heraus, dass die Wasserstoffbrückendonor-Kapazität der OH-Gruppe aufgrund der guten Aktivitäten der Methoxyderivate keine Relevanz für hohe Bindungsaffinitäten hat. Glu282 (der potentielle Wasserstoffbrückenakzeptor) bildet offensichtlich keine Interaktionen mit den Hemmstoffen aus. Der Einfluss dieser Aminosäure auf die Ligandbindung wird ohnehin kontrovers diskutiert. Während Mutagenesestudien keine signifikante Interaktion zwischen Glu282 und der 3-OH Gruppe des Substrates anzeigten [Puranen 1997], wurden in diversen unabhängigen Kristallstrukturen Wasserstoffbrückenbindungen zwischen beiden gefunden [Azzi 1996, Sawicki 1999].

Die Wasserstoffbrückenakzeptorkapazität des Thiophen-verknüpften Phenols könnte eine etwas wichtigere Rolle spielen, da die unsubstituierte Verbindung **III.1** die 17β-HSD1 schächer hemmt als die hydroxylierten Analoga. Der potentielle Interaktionspartner (Wasserstoffbrückendonor) ist in diesem Fall das His221, das zum einen als wichtiger Faktor

für Substratinteraktionen bekannt ist [Mazza 1998], zum anderen aber auch beschrieben ist, über intramolekulare Wasserstoffbrücken mit Glu282 an der Enzymstabilisierung beteiligt zu sein [Mazumdar 2009].

Da am Thiophen-verknüpften Phenylring keine direkt angebundene funktionelle Gruppe für eine hohe inhibitorische Aktivität zwingend erforderlich ist, wurde in einem weiteren Schritt untersucht, wie viel Platz innerhalb dieser C-terminalen Region für Substituenten zur Verfügung steht. Während 2fach- (**III.6**) und 3fach- (**III.7** und **III.8**) methoxylierte Derivate einen starken Abfall an Aktivität zeigten, stellte sich bei den mono-methoxylierten bzw. mono-ethoxylierten Verbindungen heraus, dass die *para*-Position am wenigsten dazu geeignet ist, um sterisch anspruchsvolle Substituenten einzuführen (Tabelle 6).

Tabelle 6: *Hemmung der 17β-HSD1 und 17β-HSD2 durch methoxy-Verbindungen*

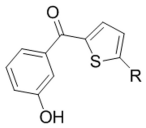


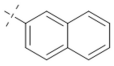
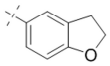
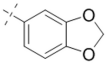
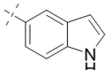
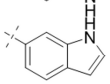
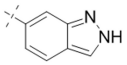
Verb.	R	IC_{50} [nM][a]		SF[d]
		17β-HSD1[b]	17β-HSD2[c]	
III.1	H	155	128	0,8
III.2	2-methoxy	86	196	2
III.3	3-methoxy	95	342	4
III.4	4-methoxy	220	507	2
III.6	3,4-dimethoxy	44[e]	44[e]	
III.7	3,4,5-trimethoxy	54[e]	26[e]	
III.8	2,3,4-trimethoxy	515	915	2

[a] Mittelwert aus drei Messungen, Standardabweichung < 14 % (außer IC_{50} (17β-HSD1) von **III.8**: < 26 %)
[b] Humane Plazenta, cytosolische Fraktion, Substrat [^{3}H]-E1, 500 nM, Cofaktor NADH, 500 µM
[c] Humane Plazenta, microsomale Fraktion, Substrat [^{3}H]-E2, 500 nM, Cofaktor NAD^+, 1500 µM
[d] SF = Selektivitätsfaktor = IC_{50} (17β-HSD2) / IC_{50} (17β-HSD1)
[e] % Hemmung bei Inhibitorkonzentration von 1 µM

Die enge räumliche Begrenzung um diesen Phenylring wird auch durch den Vergleich von Verbindung **III.6** und deren rigidifiziertem Derivat **III.26** offensichtlich, da letzteres trotz der geringen Veränderung eine vielfach höhere Affinität zur 17β-HSD1 zeigt. Generell haben Hemmstoffe, bei denen der Phenylring gegen ein kondensiertes Ringsystem ausgetauscht ist, ein gutes inhibitorisches Profil (siehe vor allem bei den sauerstoffhaltigen **III.25** und **III.26** in Tabelle 7). Lediglich das Naphthalen-Derivat **III.24** erreicht einen etwas höheren IC_{50}-Wert von 300 nM, wobei man nicht genau festlegen kann, ob die reduzierte Aktivität durch die Größe des Naphthalens oder durch das Fehlen eines Heteroatoms begründet ist.

Tabelle 7: *Hemmung der 17β-HSD1 und 17β-HSD2 durch Verbindungen mit kondensierten Ringen*

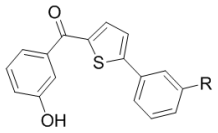


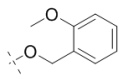
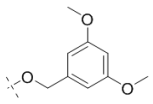
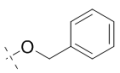
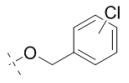
Verb.	R	IC_{50} [nM][a]		SF[d]
		17β-HSD1[b]	17β-HSD2[c]	
II.24		300	253	0,8
II.25		55	319	6
II.26		66	299	5
II.27		151	173	1
II.28		192	283	2
II.29		107	410	4

[a] Mittelwert aus drei Messungen, Standardabweichung unter 23 %
[b] Humane Plazenta, cytosolische Fraktion, Substrat [^{3}H]-E1, 500 nM, Cofaktor NADH, 500 µM
[c] Humane Plazenta, microsomale Fraktion, Substrat [^{3}H]-E2, 500 nM, Cofaktor NAD^+, 1500 µM
[d] SF = Selektivitätsfaktor = IC_{50} (17β-HSD2) / IC_{50} (17β-HSD1)

Im Hinblick auf flexiblere Substituenten stellte sich die *meta*-Position des Thiophen-verknüpften Phenylringes in Bezug auf Hemmung der 17β-HSD1 und Selektivität gegenüber der 17β-HSD2 als vorteilhaft heraus. Interessanterweise führte eine Vergrößerung des Substituenten in dieser Studie sukzessiv zu einer verbesserten Selektivität (Selektivitätsfaktor (SF) von **III.3** < **III.10** < **III.12** < **III.13**, **III.16-III.19**).

Tabelle 8: *Einfluss der Größe des Substituenten*
(SF = Selektivitätsfaktor = IC_{50} (17β-HSD2) / IC_{50} (17β-HSD1))



Verb.	R	SF	Verb.	R	SF
III.3		4	**III.13**		> 22
III.10		6	**III.16**		
III.12		13	**III.17-III.19**		

Die IC_{50}-Werte aller Benzyloxy-Derivate liegen im gleichen Bereich (zwischen IC_{50} = 30 und 60 nM) und zeigen keine großen Unterschiede zu dem Wert der *meta*-Ethoxy-Verbindung **III.10** (IC_{50} = 78 nM). Daher scheint der Phenylring des Benzyloxysubstituenten keine spezifischen Interaktionen mit der 17β-HSD1 einzugehen, während er jedoch die Selektivität gegenüber dem Typ 2 Enzym (Tabelle 8) beeinflusst. Möglicherweise könnte der Phenylring sogar aus dem 17β-HSD1 Enzym herausragen, was dessen geringen bzw. fehlenden Einfluss auf die Hemmaktivität begründen würde. In dem Fall müsste diese Enzymregion sowohl der 17β-HSD1 als auch der 17β-HSD2 genauer untersucht und verglichen werden, um Einblicke hinsichtlich des Selektivitätsgewinns zu bekommen.
Verbindung **III.23**, die mit ihrem Methylbenzensulfonamidrest ebenfalls einen Phenylring in dieser Position besitzt, ist der bislang aktivste Hemmstoff dieser Substanzklasse (IC_{50} = 12 nM), der keine zwei Hydroxygruppen trägt. Verglichen mit ihrem Methylsulfonamid-Analogon **III.21** (IC_{50} = 150 nM) führt der zusätzliche Phenylring zu einer 12fach höheren Aktivität. Einige steroidale Hemmstoffe, die ebenfalls eine flexible Seitenkette mit Phenylring haben (welcher laut Molekularen Docking Studien in vergleichbarer Position bindet), sind bereits als hochpotente Inhibitoren der 17β-HSD1 beschrieben [Schering 2006a, 2006b]. Beim Vergleich der Seitenketten und den vermuteten Bindungspositionen der steroidalen Hemmstoffe, der Benzyloxy-Derivate und Verbindung **III.23** scheint der Phenylring stets in die gleiche Region des Enzyms zu ragen. Eine molekulare Docking Studie mit **III.23** bestätigte diese Vermutung und ergab die gleichen Interaktionen für den gesamten Benzoylrest, ausgenommen der zweiten Wasserstoffbrückenbindung des Keton-Sauerstoffs mit Ser142, die bei dieser Pose nicht mehr gefunden wurde. Der Methylbenzensulfonamidrest interagiert mit 2 Aminosäuren der C-terminalen Region: His221 bildet eine Wasserstoffbrücke mit dem Sulfonamid-Sauerstoff und Phe259 interagiert über π-π Wechselwirkungen (T-shaped) mit dem Benzen. Die fehlende Wasserstoffbrücke der Ketogruppe wird offensichtlich durch die zusätzlichen π-π Interaktionen der Seitenkette mit Phe259 ausgeglichen.

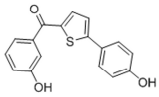
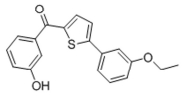
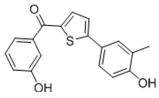
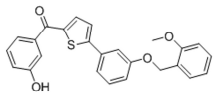
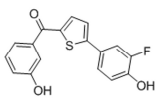
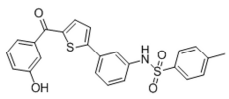
Die Wirkstoffkandidaten mit dem besten inhibitorischen Profil wurden hinsichtlich ihrer Affinität zu den Estrogenrezeptoren ERα und ERβ, ihrer intrazellulären Hemmaktivität in T47D Zellen sowie ihrer metabolischen Stabilität unter Verwendung humaner Lebermikrosomen evaluiert.
Um das Risiko für unerwünschte Nebenwirkungen so gering wie möglich zu halten, sollten potentielle Hemmstoffe keine oder nur geringe Affinität zu den ERs haben, um somit zumindest intrinsische estrogene Effekte zu vermeiden. Bei dem durchgeführten kompetitiven Bindungsassay wird die relative Bindungsaffinität (RBA) von E2 gleich 100 % gesetzt. Da E1 eine 10fach geringere Affinität zum ERα [Zhu 2006] sowie eine 50fach geringere zum ERβ [Zhu 2006] hat, und E1 in der Zelle ubiquitär vorkommt, werden alle Verbindungen, die

bei diesem Test einen RBA-Wert unter 0,1 % erreichen, als ohne relevante Affinität für die *in vivo*-Ausübung estrogener Effekte eingestuft. Innerhalb dieser Substanzklasse zeigten alle getesteten Substanzen eine niedrigere RBA als 0,1 %, was unsere Strategie der Entwicklung nichtsteroidaler Inhibitoren bekräftigt.

Im Hinblick auf die intrazelluläre inhibitorische Aktivität zeigt die Substanzklasse der bicyclisch substituierten Hydroxyphenylmethanone hervorragende Resultate, auch im Vergleich mit den Hemmstoffen aus der Klasse der Bis(hydroxyphenyl)heterocyclen. Während die potentesten Substanzen letztgenannter Verbindungsklasse intrazellulär lediglich IC_{50}-Werte um 300 nM aufweisen [Bey 2009], zeigen Vertreter der neuen Klasse IC_{50}-Werte im niedrigen nanomolaren Bereich (Tabelle 9). Die zusätzliche Keto-Funktion scheint daher einen positiven Einfluss auf die Zellpermeabilität und/oder den intrazellulären Metabolismus zu haben. Im Rahmen der bicyclisch substituierten Hydroxyphenylmethanone fällt auf, dass Hemmstoffe mit zwei Hydroxygruppen bessere Hemmwerte erzielen als Verbindungen, die am Thiophen-verknüpften Phenylring andere Substituenten tragen. Bei Letzteren können lediglich **III.10** und **III.23** ihre guten IC_{50}-Werte aus dem zellfreien Assay auch in T47D Zellen annähernd bestätigen. Vor allem Benzyloxyverbindungen (z.B. **III.13**) zeigten intrazellulär einen starken Abfall an Aktivität, wobei man hier eine schnelle intrazelluläre Metabolisierung als Grund vermuten könnte.

Die getesteten Verbindungen mit zwei Hydroxygruppen stellen hochaktive intrazelluläre Inhibitoren der 17β-HSD1 dar, was sie zu vielversprechenden Wirkstoffkandidaten macht.

Tabelle 9: *Hemmung der 17β-HSD1 durch ausgewählte Verbindungen in zellulären und zellfreien Assay*

Verb.	Struktur	IC_{50} [nM][a] zellfreier Assay[b]	IC_{50} [nM][a] zellulärer Assay[c]	Verb.	Struktur	IC_{50} [nM][a] zellfreier Assay[b]	IC_{50} [nM][a] zellulärer Asaay[c]
II.4		33	30	**III.10**		78	154
II.14		8	27	**III.13**		35	441
II.15		19	17	**III.23**		12	78

[a] Mittelwert aus drei Messungen, Standardabweichung unter 24 %
[b] Humane Plazenta, cytosolische Fraktion, Substrat [^{3}H]-E1, 500 nM, Cofaktor NADH, 500 µM
[c] T47D Zellen, Substrat [^{3}H]-E1, 50 nM

Einen Unterschied zwischen Verbindungen mit zwei Hydroxygruppen und solchen mit nur einer kann man auch bei der metabolischen Stabilität feststellen. In diesem Fall zeigen Vertreter der Substanzen mit einer OH-Funktion eine hohe intrinsische Clearance und dementsprechend

kurze Halbwertszeiten von ca. 15 Minuten (z.B. 12,8 Minuten für Verbindung **III.23**). Die getesteten Verbindungen mit zwei OHs liegen dagegen im Bereich einer mittleren Clearance mit Halbwertszeiten im Bereich von 95 Minuten (z.B. 100 Minuten für Verbindung **II.14**) und belegen damit ihre geringere Anfälligkeit für einen metabolischen Abbau. Aufgrund ihres ausgezeichneten metabolischen Stabilitätsprofils bieten sich jedoch vor allem diese dihydroxylierten Verbindungen für die Durchführung einer umfassenden Pharmakokinetikstudie an, bei dem auch der Phase 2-Metabolismus erfasst wird.

5 ZUSAMMENFASSUNG

Die vorliegende Arbeit beschreibt die Kombination eines struktur- und ligandbasierten Designkonzeptes, welches zur Entwicklung einer neuen Substanzklasse von hochaktiven und selektiven Hemmstoffen der 17β-HSD1 führt, den bicyclisch substituierten Hydroxyphenylmethanonen.

Als Estron-Mimetika entwickelt, bildeten die 20 synthetisierten Verbindungen der heterocyclisch substituierten Biphenylole sowie die Ergebnisse molekularer Docking Studien derer potentesten Vertreter die Grundlage für das Design der neuen Hemmstoffklasse. Das Einschließen einer zusätzlichen, überwiegend lipophilen Bindetasche unterhalb des katalytischen Zentrums in ein neues 3-Punkt-Pharmakophor-Modell stellte den Startpunkt zur Synthese und Weiterentwicklung von mehr als 55 Derivaten der bicyclisch substituierten Hydroxyphenylmethanone dar. Neben einer sehr starken Hemmung der 17β-HSD1 im z.T. einstelligen nanomolaren Bereich zeigen die potentesten Vertreter, dem therapeutischen Konzept entsprechend, eine hohe Selektivität gegenüber dem biologischen Gegenspieler 17β-HSD2 (bis zu 50fach) sowie keine relevante Affinität gegenüber den beiden Estrogenrezeptoren ERα und ERβ. Im Gegensatz zu den bereits bekannten Substanzklassen können diese Inhibitoren ihre starke Hemmung, detektiert im zellfreien Assay, zusätzlich in einem zellbasierten Assay mit der Brustkrebszelllinie T47D mit IC_{50}-Werten im unteren nanomolaren Bereich, bestätigen. Einige Verbindungen wurden hinsichtlich ihrer metabolischen Stabiltät mit humanen Lebermikrosomen evaluiert und ergänzen mit hohen bis mittleren intrinsischen Clearance-Werten, welche alle zwischen denen der marktbekannten Wirkstoffe Dextromethorphan und Verapamil lagen, ihre bislang sehr guten präklinischen Werte.

Die pharmakokinetische Evaluierung von Verbindung **II**.**14** sowie deren Einsatz in einem krankheitsorientierten Mausmodell, welches die *in vivo*-Wirksamkeit beweisen soll, wird aktuell durchgeführt. Bei einem positiven Verlauf werden weitere präklinische Tests unternommen.

6 SUMMARY

The present work describes the combination of a structure- and ligandbased design concept, which led to the development of a new class of highly active and selective 17β-HSD1 inhibitors, namely bicyclic substituted hydroxyphenylmethanones.

Designed as estrone-mimetics, 20 synthesized compounds as well as molecular docking results of their most potent representatives provide the basis for the design of the new inhibitor class. The inclusion of an additional rather lipophilic subpocket, located beneath the catalytic center, in a new 3-point-pharmacophore model was the starting point for the synthesis and development of more than 55 inhibitors in the class of bicyclic substituted hydroxyphenylmethanones. Besides a strong 17β-HSD1 inhibition in the very low nanomolar range, the most potent compounds show high selectivity toward the biological counterpart 17β-HSD2 (up to 50 fold). Furthermore, they reveal neglectable affinity towards the estrogen receptors ERα and ERβ, according the therapeutic concept. In contrast to the already known compound classes, these inhibitors confirmed their strong inhibition, detected in the cell-free assay, in the breast cancer cell line T47D with IC_{50}-values in the low nanomolar range. Metabolic stability of the most promising compounds was evaluated using human liver microsomes. Summarizing, these compounds reveal so far very good preclinical results with high to middle intrinsic clearances between the values of the marketed drugs dextromethorphan and verapamil.

The pharmacokinetic evaluation of compound **II**.**14** as well as its application in a disease-oriented mouse-model, which should prove its *in vivo* efficacy, is currently in progress. In case of positive results, further preclinical tests will be accomplished.

7 ANHANG

7.1 Abkürzungsverzeichnis

7.1.1 Allgemeines

17β-HSD	17β-Hydroxysteroid-Dehydrogenase
Å	Ångström
AKR	Aldo-Keto Reduktase
APCI	atmospheric pressure chemical ionisation
CC	Säulenchromatographie
$CDCl_3$	deuteriertes Chloroform
CD_3COCD_3	deuteriertes Aceton
CD_3SOCD_3	deuteriertes DMSO
CD_3OD	deuteriertes Methanol
cmpd	compound
COX	Cyclooxygenase
CYP	Cytochrom P450
DFT	density functional theory
DME	Dimethoxyethan
DMSO	Dimethylsulfoxid
E1	Estron
E2	Estradiol
EDTA	Ethylendiamintetraessigsäure
eq	Äquivalent
ER	Estrogenrezeptor
ESI	electrospray ionisation
EtOH	Ethanol
FDA	food and drug administration
GnRH	Gonadotropin Releasing Hormon
HPLC	high performance liquid chromatography
HSD	Hydroxysteroid-Dehydrogenase
Hz	hertz
IC_{50}	benötigte Konzentration für 50 % Hemmung
IUPAC	international union for pure and applied chemistry
MEP	molecular electrostatic potential
mRNA	messenger Ribonukleinsäure
MS	Massenspekrometrie
MW	Mikrowelle
NAD^+	Nicotinsäureamid-Adenin-Dinukleotid
NADH	Nicotinsöureamid-Adenin-Dinukleotid (reduzierte Form)
$NADP^+$	Nicotinsäureamid-Adenin-Dinucleotidphosphat
NADPH	Nicotinsäureamid-Adenin-Dinucleotidphosphat (reduzierte Form)
NMR	nuclear magnetic resonance
PDB-ID	Proteindatenbank-Identifikationscode
PGE_2	Prostaglandine E_2
ppm	parts per million
RBA	relative Bindungsaffinität
RMSD	root mean square distance
rt	room temperature

SAR	Struktur-Wirkungsbeziehung
SDR	Short-Chain Dehydrogenase/ Reduktase
SERM	selektiver Estrogenrezeptor Modulator
SF	Selektivitätsfaktor
T	Testosteron
TE	Tris-EDTA
THF	Tetrahydrofuran
TLC	Dünnschichtchromatographie
W	Watt
WW	Wechselwikungen

7.1.2 Aminosäuren

R	Arg	Arginin
N	Asn	Asparagin
D	Asp	Asparaginsäure
E	Glu	Glutaminsäure
H	His	Histidin
L	Leu	Leucin
K	Lys	Lysin
M	Met	Methionin
F	Phe	Phenylalanin
P	Pro	Prolin
S	Ser	Serin
Y	Tyr	Tyrosin
V	Val	Valin

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