GEZIELTE EINGRIFFE IN DIE STEROIDBIOSYNTHESE DURCH CYP INHIBITOREN ZUR ENTWICKLUNG NEUARTIGER THERAPEUTIKA FÜR DIE BEHANDLUNG VON PROSTATAKARZINOM UND METABOLISCHEM SYNDROM

Dissertation

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von

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I THINK PERHAPS THE MOST IMPORTANT PROBLEM IS THAT WE ARE TRYING TO UNDERSTAND THE FUNDAMENTAL WORKINGS OF THE UNIVERSE VIA A LANGUAGE DEVISED FOR TELLING ONE ANOTHER WHERE THE BEST FRUIT IS.

TERRY PRATCHETT

MEINEN ELTERN

ZUSAMMENFASSUNG

An der menschlichen Steroid-Biosynthese sind sechs CYP Enzyme beteiligt. Vier davon stellen sehr interessante Targets zur Entwicklung neuer Medikamente für die Therapie einer Reihe von hormonabhängigen Krankheiten dar. In dieser Arbeit wurden zwei Targets ausgewählt, zum einen das Schlüsselenzym der Androgenbiosynthese, CYP17, und zum anderen CYP11B1, welches den entscheidenden Schritt in der Cortisolbiosynthese darstellt. Design und Synthese der vorgestellten CYP17 Inhibitoren, die zur Therapie des hormonabhängigen Prostatakarzinoms dienen können, wurden ausgehend von bekannten biphenylischen Grundstrukturen entwickelt. Die dabei entstandenen Inhibitoren konnten, mit IC_{50} Werten bis zu 52 nM, sowohl bezüglich ihrer Aktivität zum Target CYP17 als auch hinsichtlich ihrer Selektivität gegenüber einer Reihe von weiteren CYP Enzymen stark optimiert werden. Die vorgestellten CYP11B1 Inhibitoren wurden ausgehend von einem Etomidat-basierten Design Konzept entwickelt. Die so gefundenen CYP11B1 Inhibitoren stellen die ersten selektiven Hemmstoffe dieses Enzyms dar. Eine besondere Herausforderung bei der Optimierung dieser Verbindungen war die Selektivität zum mit 93 % hochhomologen CYP11B2. Mit bis zu 49facher Selektivität gegenüber CYP11B2 und gleichzeitig hoher Hemmaktivität gegenüber CYP11B1 mit IC_{50} Werten im bis zu einstelligen nanomolaren Bereich war der durchgeführte Ansatz erfolgreich. Nach erweiterten pharmakologischen Untersuchungen und gegebenenfalls erforderlichen strukturellen Optimierungen sollen aus den vorgestellten Substanzklassen Entwicklungskandidaten hervorgehen.

ABSTRACT

Six CYP enzymes are involved in human steroid biosynthesis. Four of them constitute very interesting targets for the development of new drugs in order to treat a variety of hormone-dependent diseases. Herein, two targets were selected: on the one hand, CYP17, the key-enzyme in androgen biosynthesis and on the other hand CYP11B1, catalyzing the main step in cortisol biosynthesis. Design and synthesis of the investigated CYP17 inhibitors, which should be promising drugs in the treatment of hormone dependent prostate cancer were developed outgoing from known biphenylic core-structures. Optimization of these compounds yielded inhibitors with IC_{50} values as low as 52 nM, which showed strong activity toward the target CYP17 as well as high selectivity toward a broad range of further CYPs. The herein presented CYP11B1 inhibitors were developed outgoing from an etomidate-based design concept and are the first selective inhibitors of this enzyme. Particularly challenging was the optimization of these compounds regarding their selectivity toward the 93 % homologuos CYP11B2. With up to 49fold selectivity toward CYP11B2 and at the same time high inhibitory activity toward CYP11B1 in the low nanomolar range, the development was successful. In conclusion, after extended pharmacological testing, and, if required, further structural optimisation, the presented class of substances should give rise to new developmental candidates.

EINGEFLOSSENE PUBLIKATIONEN

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

I **Novel CYP17 inhibitors: Synthesis, biological evaluation, structure-activity relationships and modelling of methoxy- and hydroxy-substituted methyleneimidazolyl biphenyls**
Ulrike E. Hille, Qingzhong Hu, Carsten Vock, Matthias Negri, Marc Bartels, Ursula Müller-Vieira, Thomas Lauterbach and Rolf W. Hartmann
Eur. J. Med. Chem. **2008***, 51,* 5064 – 5074.

II **CYP17 Inhibitors. Annulations of Additional Rings in Methylene Imidazole Substituted Biphenyls: Synthesis, Biological Evaluation and Molecular Modelling**
Mariano A.E. Pinto Bazurco Mendieta, Matthias Negri, Qingzhong Hu, Ulrike E. Hille, Carsten Jagusch, Kerstin Jahn-Hoffmann, Ursula Müller-Viera, Dirk Schmidt, Thomas Lauterbach and Rolf W. Hartmann
Arch. Pharm Chem. Life Sci. **2008**, *341*, 597 – 609.

III **Steroidogenic cytochrome P450 (CYP) enzymes as drug targets: Combining substructures of known CYP inhibitors leads to compounds with different inhibitory profile**
Ulrike E. Hille, Qingzhong Hu, Mariano A.E. Pinto-Bazurco Mendieta, Marc Bartels, Carsten A.Vock, Thomas Lauterbach and Rolf W. Hartmann
C.R.Chimie **2009**, *12*, 1117 – 1126.

IV **Discovery of the first selective steroid-11β-hydroxylase (CYP11B1) inhibitors for the treatment of cortisol dependent diseases**
Ulrike E. Hille[†], Christina Zimmer[†], Carsten A. Vock and Rolf W. Hartmann
Med. Chem.Lett. **2010**, accepted
[†] *These authors contributed equally to this work*

V **Optimization of the first selective steroid-11β-hydroxylase (CYP11B1) inhibitors for the treatment of cortisol dependent diseases**
Ulrike E. Hille, Christina Zimmer, Jörg Haupenthal and Rolf W. Hartmann
Med. Chem.Lett. **2010**, submitted

STELLUNGNAHME ÜBER DIE BEITRÄGE DER AUTORIN

Die Autorin möchte zu ihren Beiträgen zu den Veröffentlichungen **I** – **V** in der Dissertation stellungnehmen.

I Die Autorin lieferte wesentliche Beiträge zum Design Konzept der Inhibitoren und war für die Planung, Synthese und Charakterisierung der meisten Verbindungen verantwortlich. Verbindungen **1** – **2** wurden von Dr. Marc Bartels synthetisiert. Verbindungen **13** – **15** wurden von Qingzhong Hu synthetisiert. Die Autorin war verantwortlich für die Interpretation der Ergebnisse und konzipierte und verfasste das Manuskript.

II Die Autorin lieferte wesentliche Beiträge zum Design Konzept der Inhibitoren und war für die Planung, Synthese und Charakterisierung der Verbindungen **4**, **5** und **19** – **21** verantwortlich. Verbindungen **1** – **3** und **6** – **19** wurden von Dr. Kerstin Jahn-Hofmann und Dr. Mariano Pinto synthetisiert. Die Autorin trug zur Interpretation der Ergebnisse und zur Erstellung des Manuskriptes bei.

III Die Autorin lieferte wesentliche Beiträge zum Design Konzept der Inhibitoren. Verbindungen **1** und **2** wurden von Qingzhong Hu synthetisiert. Verbindung **3** wurde von Dr. Mariano Pinto synthetisiert. Verbindungen **4** – **7** wurden von Dr. Marc Bartels synthetisiert. Die Autorin war verantwortlich für die Interpretation der Ergebnisse und konzipierte und verfasste das Manuskript.

IV Die Autorin entwickelte das Design Konzept der Inhibitoren und war für die Planung, Synthese und Charakterisierung der meisten Verbindungen verantwortlich. Verbindungen **13** – **15** wurden von Dr. Carsten Vock synthetisiert. Verbindungen **34** – **36** wurden von Dr. Marc Bartels synthetisiert. Die Autorin war verantwortlich für die Interpretation der Ergebnisse und konzipierte und verfasste das Manuskript.

V Die Autorin entwickelte das Design Konzept der Inhibitoren und war für die Planung, Synthese und Charakterisierung aller Verbindungen verantwortlich. Die Autorin war verantwortlich für die Interpretation der Ergebnisse und konzipierte und verfasste das Manuskript.

Desweiteren sind in die vorliegende Arbeit noch folgende vier Publikationen eingeflossen:

A **Synthesis, biological evaluation and molecular modeling studies of novel ACD- and ABD-ring steroidomimetics as inhibitors of CYP17**

Mariano A.E. Pinto Bazurco Mendieta, Matthias Negri, Carsten Jagusch, Ulrike E. Hille, Dirk Schmidt, Klaus Hansen and Rolf W. Hartmann

Bioorg. Med. Chem.Lett. **2008**, *18*, 267 – 273.

B **Synthesis, biological evaluation and molecular modeling studies of methyleneimidazole substituted biaryls as inhibitors of human 17 α-hydroxylase-17,20-lyase (CYP17) – Part I: heterocyclic modifications of the core structure**

Carsten Jagusch, Matthias Negri, Ulrike E. Hille, Qingzhong Hu, Marc Bartels, Kerstin Jahn-Hoffmann, Mariano A. E. Pinto-Bazurco Mendieta, Klaus Biemel, B Rodenwaldt, Ursula Müller-Vieira, Dirk Schmidt, Thomas Lauterbach, Maurizio Recanatinti, Andrea Cavalli and Rolf W. Hartmann

Bioorg.Med.Chem. **2010**, *16*, 1992 – 2010

C **i-Propylidene substitution increases activity and selectivity of biphenyl methylene 4-pyridine type CYP17 inhibitors**

Qingzhong Hu, Lina Yin, Carsten Jagusch, Ulrike E. Hille and Rolf W. Hartmann

J. Med.Chem. **2010**, submitted.

D **Replacement of imidazolyl by pyridyl in biphenyl methylenes results in selective CYP17 and dual CYP17 / CYP11B1 inhibitors for the treatment of prostate cancer**

Qingzhong Hu, Carsten Jagusch, Jörg Haupenthal, Ulrike E. Hille and Rolf W. Hartmann

J. Med.Chem. **2010**, submitted.

ABKÜRZUNGSVERZEICHNIS

ABT	Abirateron
ACTH	Adrenokortikotropes Hormon
CYP	Cytochrom P450
CYP11A1	Cholesterol Desmolase
CYP11B1	11β-Hydroxylase
CYP11B2	Aldosteron Synthase
CYP17	17α-Hydroxylase-17,20-lyase
CYP19	Aromatase
Et	Ethyl
FSH	Follikel stimulierendes Hormon
GnRH	Gonadotropin Releasing Hormon
Het	Heteroaryl
HSD	Hydroxysteroiddehydrogenase
IC_{50}	Konzentration, die zur 50 %igen Hemmung benötigt wird
*i*Pr	Isopropyl
KTZ	Ketoconazol
LH	Luteinisierendes Hormon
Me	Methyl
µM	Mikromolar
NADP	Nicotinamidadenindinukleotidphosphat
nM	Nanomolar
Ph	Phenyl
PK	Prostata Karzinom
PSA	Prostata-spezifisches Antigen

INHALT

1 Einleitung

1.1 CYP Enzyme

1.1.1 Vorkommen und Funktion

Die Cytochrom P450 Enzyme, die eine große Familie von Hämproteinen bilden, kommen ubiquitär in Pflanzen, Tieren und Bakterien vor. Die Entwicklung der nahezu 500 bekannten CYP450 Gene erfolgte aus einem gemeinsamen Vorläufergen, welches vor etwa 3,5 Milliarden Jahren entstanden sein dürfte. Die Funktion dieser Enzyme besteht in der Katalyse endogener Synthesewege und dem oxidativen Abbau von Xenobiotika. Sie sind die Hauptenzyme im Phase I Metabolismus einer Reihe von endogenen und exogenen Substanzen.[1,2] Die Benennung P450 resultiert aus der Absorption des membrangebundenen reduzierten Cytochrom P450-CO Komplexes, wobei das P für Pigment steht und 450 für die Wellenlänge des Absorptionsmaximums in nm.[3,4] Die Einteilung der CYP Enzyme erfolgt nach ihrer Sequenzhomologie in Familien, z.B. CYP11 (Sequenzhomologie > 40 %) und Subfamilien, z.B. CYP11B (Sequenzhomologie > 55%). Die einzelnen Isoenzyme der Subfamilien werden mit laufenden Nummern bezeichnet, z. B. CYP11B1 und CYP11B2. Hierbei ist zu erwähnen, dass die Enzyme der Familien CYP1 bis CYP3 hauptsächlich für die Verstoffwechselung von Xenobiotika zuständig sind, während CYP4 bis CYP51 dem Metabolismus endogener Substrate zugeordnet werden.[5,6]

Beim Menschen findet man die höchste Konzentration der Cytochrom P450 Enzyme in der Leber, daneben kommen sie auch in extrahepatischen Geweben wie Lunge, Darm, in Fortpflanzungsorganen und endokrinen Geweben vor. Während man bei einigen Bakterienarten auch lösliche Formen findet, sind die CYP Enzyme im menschlichen Körper immer membrangebunden. Die Betrachtung der subzellulären Ebene zeigt eine Membranverankerung am endoplasmatischen Retikulum und der inneren Mitochondrienmembran über eine transmembranäre N-terminale Helix und die pre-A und F-G Schleife.[7]

Funktionell gesehen katalysieren die CYP Enzyme ein breites Spektrum an Reaktionen. Neben Oxidationen von gesättigten Kohlenwasserstoffen, Olefinen, Aromaten und Heteroatomen (N, S) sind sie in der Lage Dehalogenierungen, N- und O- Dealkylierungen, Spaltung von C-C-Bindungen, sowie Dehydrogenierungen und Isomerisierungen durchzuführen. Sie werden als mischfunktionelle Oxidasen bezeichnet, da sie ein Atom molekularen Sauerstoffs in ihr Substrat einbauen, während das andere Sauerstoffatom zu Wasser reduziert wird. Für die Aktivierung des molekularen Sauerstoffs werden Elektronen benötigt, die über ein Elektronentransfersystem geliefert werden. Dieses System

spielt deshalb eine Rolle, da für die Reaktion zwei Elektronen aus NADPH geliefert werden, jedoch im Falle der meisten CYP Enzyme immer nur eines aufgenommen werden kann. Hinsichtlich des Elektronentransfers kann man die CYP Enzyme in vier Klassen mit zwei hauptsächlich vorkommenden Systemen einteilen: Typ 1 kommt in den Mitochondrien vor und erhält seine Elektronen von NADPH über Ferredoxin Reduktase und Ferredoxin, während Typ 2 die Enzyme des glatten ER, wie z.B. CYP17, beinhaltet, deren Elektronentransfer vom NADPH über P450-Oxido-Reduktase und Cytochrom b_5 stattfindet. Daneben findet man noch seltener Typ 3 ohne externes Elektronentransfersytem bei Enzymen, die Isomerisierungen katalysieren und Enzyme des Typs 4, die, wie im Falle der bakteriellen Stickstoffmonoxidreduktase, die Elektronen direkt über NAD erhalten.[8]

Die katalysierte Reaktion ist der Einschub eines Atoms aus molekularem Sauerstoff in ein organisches Substrat (R–H), wobei das andere Sauerstoffatom zu Wasser reduziert wird:

$$R\text{–}H + O_2 + NADPH + H^+ \longrightarrow R\text{–}OH + H_2O + NADP^+$$

Diese Reaktion unterliegt einem katalytischen Kreislauf. Im Ruhezustand koordiniert Fe(III) an das Porphyrin, das Thiolat des verankernden Cysteins und ein axiales Wassermolekül (Abb.1, A). Letzteres wird zu Beginn der Reaktion durch das Substrat verdrängt (B).

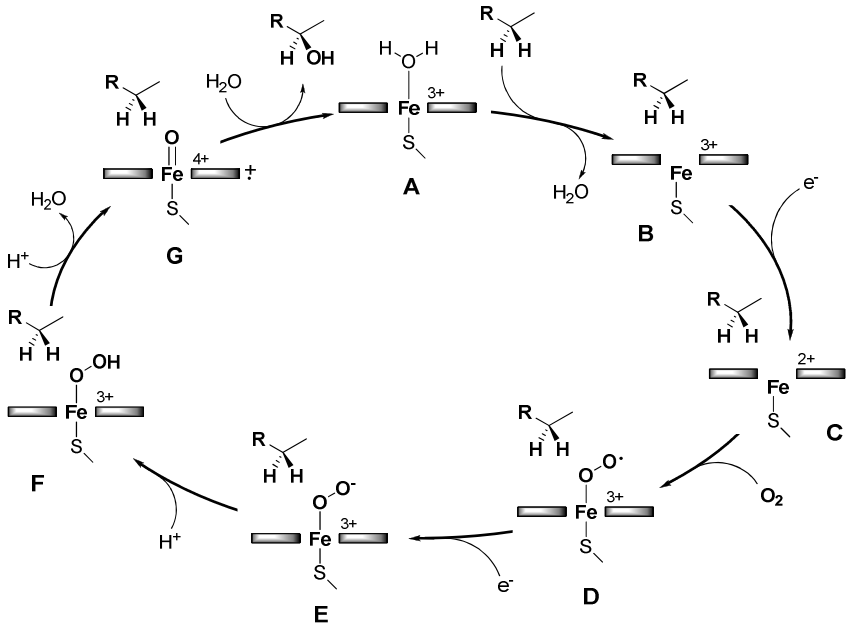

Abbildung 1. Katalytischer Kreislauf der P450 Enzyme[9]

Dabei wird das Eisen aus der planaren Ebene des Porphyrins verschoben, unterliegt einer Spinänderung (von low spin zu high spin) und kann so aufgrund des veränderten Redoxpotentials Elektronen vom Cofaktor NADPH über das Elektronentransfersystem aufnehmen. Das Eisen wird damit zu Fe(II) reduziert (C), woraufhin molekularer Sauerstoff unter Aufnahme eines Elektrons vom

Eisen bindet und damit formal wieder Fe(III) vorliegt (D). Nun führt ein zweiter Elektronenübertragungsschritt, der im Falle von CYP17 über Cytochrom b_5 vermittelt wird, zum formal negativ geladenen Peroxo-Komplex (E). Über eine Aminosäurenkaskade werden auf diesen Protonen übertragen (F) und nach Abspaltung eines weiteren Wassermoleküls resultiert das hochreaktive Fe(IV)-oxo-porphyrin-system (G) welches schließlich den Sauerstoff in die C-H Bindung des Substrates überträgt. Das Enzym fällt nun zurück in den Fe(III) Ruhezustand, welcher durch Aufnahme eines Wassermoleküls stabilisiert wird (A).[10]

1.1.2 CYP Enzyme in der Steroidbiosynthese

Während die meisten hepatischen CYP Enzyme relativ unspezifisch eine Reihe von strukturell sehr verschiedenen Substraten in hydrophile Abbauprodukte überführen, handelt es sich bei den CYPs der Steroidbiosynthese um Enzyme mit einer hohen Substratspezifität. Daher auch ihre Eignung als Drug Targets. In der Biosynthese sind folgende sechs CYP-Enzyme beteiligt (Abb.2): CYP11A1 (Cholesterol Side Chain Cleavage Enzyme, Desmolase), CYP21 (Steroid-21-Desmolase), CYP17 (17α-Hydroxylase-C17,20-Lyase), CYP19 (Aromatase), CYP11B1 (Steroid-11β-Hydroxylase) und CYP11B2 (Aldosteron-synthase). CYP11A1 eignet sich aufgrund seiner Beteiligung an der Bildung aller Steroide nicht als Drug Target, ebenso wie CYP21 welches an der Biosynthese von sowohl Gluco- als auch Mineralocorticoiden beteiligt ist.

CYP19 katalysiert die Umsetzung von Testosteron und Androstendion zu den Estrogenen Estron und Estradiol über eine dreifache Oxidation an C19. Die Entwicklung der Aromatase Inhibitoren zur Therapie des hormonabhängigen Brustkrebses ist wohl das bislang erfolgreichste Beispiel in der Entwicklung von CYP Hemmstoffen. Durch sie gelang es, Tamoxifen, welches durch seine Affinität zum Estrogenrezeptor eine Reihe schwerer Nebenwirkungen zeigt, als Standardtherapeutikum abzulösen.[11]

CYP17 ist innerhalb der Steroidbiosynthese an zwei Reaktionen beteiligt. Zum einen katalysiert es die Hydroxylierung von Pregnenolon und Progesteron zu ihren 17-Hydroxy-Produkten und dann in einem weiteren Oxidationsschritt die Abspaltung von Essigsäure zu den Ketonen Dehydroepiandrosteron und Androstendion. Damit stellt CYP17 den Schlüsselschritt in der Biosynthese der Androgene dar und ist somit ein vielversprechendes Target zur Entwicklung von Wirkstoffen in der Therapie des androgenabhängigen Prostatakarzinoms. Auch hier wurden bereits Hemmstoffe entwickelt, die sich in der fortgeschrittenen klinischen Prüfung befinden.[12,13]

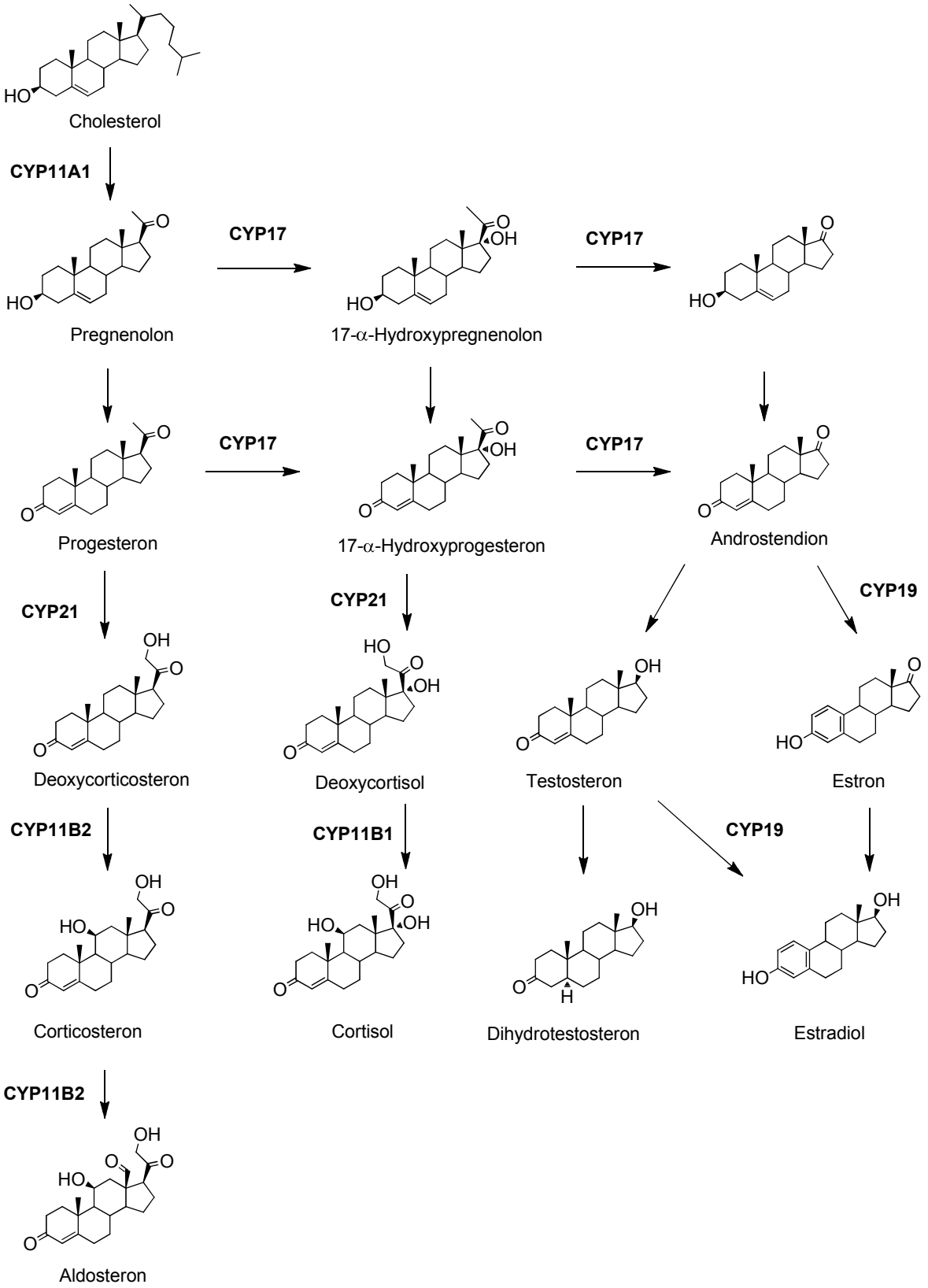

Abbildung 2: Rolle der CYP Enzyme in der Steroid-Biosynthese

Ein weiteres CYP Enzym in der Biosynthese der Steroide stellt die Aldosteron Synthase dar. Es katalysiert Oxidationen in den Positionen 11 und 18 der Steroide, also die 11-Hydroxylierung von 11-

Deoxycorticosteron zu Corticosteron und die anschließenden 18-Hydroxylierungen zu 18-Hydroxycorticosteron und weiter zu Aldosteron. Zusätzlich ist CYP11B2 in der Lage Cortisol in Position 18 zu hydroxylieren. Damit katalysiert CYP11B2 den entscheidenden Schritt in der Mineralocorticoid-Biosynthese und ist somit ein interessantes Target in der Therapie des Hyperaldosteronismus und damit in Zusammenhang stehenden Krankheiten.[14,15]

Als letztes CYP Enzym der menschlichen Steroidbiosynthese ist CYP11B1 zu nennen, welches durch Hydroxylierungen in Position 11 am Steroidgerüst in der Lage ist 11-Deoxycortisol sowie 11-Deoxycorticosteron in ihre Metaboliten Cortisol und Corticosteron umzuwandeln. Der Hauptunterschied zu CYP11B2 liegt darin, dass CYP11B1 lediglich in Position 11 hydroxyliert, während die Aldosteron Synthase Hydroxylierungen in den Positionen 11 und 18 zu katalysieren vermag. CYP11B1 ist das Schlüsselenzym der Cortisol-Biosynthese und seine selektive Hemmung ein vielversprechender Ansatz in der Therapie cortisolabhängiger Krankheiten, wie beispielsweise des Cushing-Syndroms.[16 – 19]

1.2 CYP17 als Drug Target

1.2.1 Androgenabhängiges Prostatakarzinom (PK)

Prostatakrebs gehört zu den häufigsten Krebserkrankungen des Mannes und liegt in Deutschland mit einer Neuerkrankungszahl von ca 60.000 Fällen und einem prozentualen Anteil von 26.2 % inzwischen auf Platz eins der nationalen Krebsstatistik.[20] Etwa 11.500 Männer sterben jährlich an dieser Krankheit. Die meisten Neuerkrankungen werden bei Männern ab dem 70. Lebensjahr gefunden. Die Neuerkrankungsrate liegt in dieser Altersklasse bei etwa 50%.[21] Dabei sind allerdings die postmortal diagnostizierten Prostatakarzinome noch nicht berücksichtigt. Aufgrund der sich nach oben verschiebenden Altersstruktur der Bevölkerung steigt die Bedeutung einer effizienten Prostatakrebs-Therapie. Da die Anfangsstadien des PK meist symptomlos verlaufen, findet eine Diagose häufig erst im bereits metastasierten Zustand statt. Eine lokal begrenzte Entfernung des Tumors, wie sie in den Anfangsstadien durchgeführt wird, ist dann nicht mehr möglich. Da das PK zu den wenig aggressiven Krebsformen gehört, kann oft über eine lange Zeit eine aktive Überwachung, das sogenannte „Watchful Waiting“, in Betracht kommen. Hierbei werden verschiedene Parameter wie die Höhe des PSA-Wertes (Prostata-spezifisches Antigen) und der Ploidiegrad der Krebszellen untersucht und engmaschig beobachtet.

1.2.2 Therapie des PK

Da etwa 80% aller neu diagnostizierten PK hormonabhängig sind, stellt die endokrine Therapie eine zielgerichtete Methode zur Hemmung des Wachstums von Primärtumor und Metastasen dar. Den Grundstein für diese Therapieform legten Huggins and Hodges im Jahre 1941, indem sie die Effektivität von Androgensuppression gegenüber Prostatatumoren zeigen konnten.[22] Ihre Arbeit wurde 1966 mit dem Nobelpreis für Physiologie gewürdigt. Das traditionelle Verfahren der Androgendeprivation ist die bilaterale Orchiektomie (Abb. 3), also die Absenkung der Androgenspiegel durch chirurgische Kastration. Diese stellt zwar einen schnellen und effektiven Weg der Androgensuppression dar, ist allerdings aufgrund der resultierenden psychischen Belastung nur wenig akzeptiert.

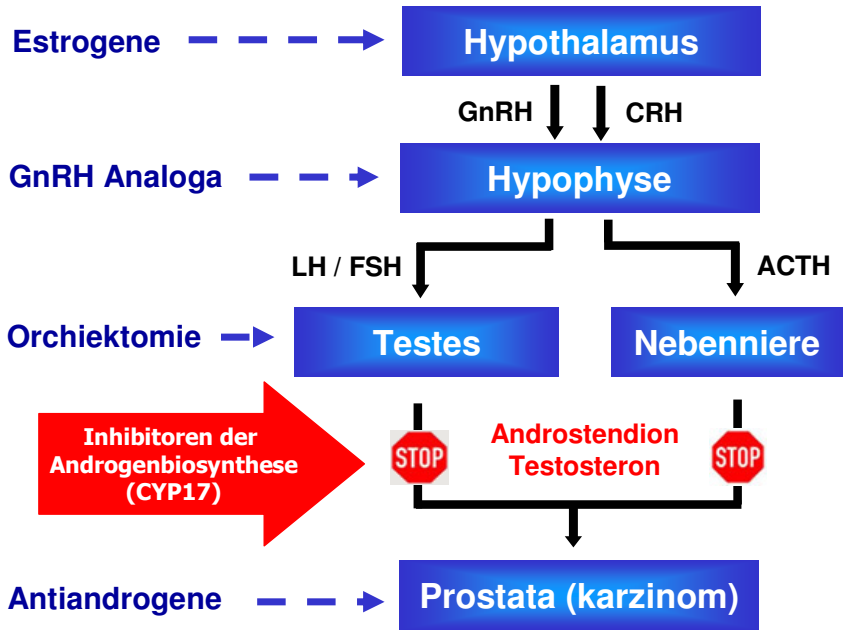

Abbildung 3: Zentrale Regulierung der Androgenproduktion und Angriffspunkte der Therapeutika zur Therapie des PK

Mit der medikamentösen Kastration durch synthetische GnRH-Analoga erreicht man eine Down-Regulation der Hypophyse und damit eine Unterdrückung der Ausschüttung von luteinisierendem Hormon (LH), wodurch in einem Zeitraum von etwa einem Monat die Testosteronlevel auf Kastrationsniveau gesenkt werden. Aufgrund eines sogenannten „Flare up"-Effekts, einer initialen Testosteronüberproduktion, müssen hier zu Beginn der Therapie zusätzlich Antiandrogene gegeben werden.[23]

Beiden Therapieformen gemeinsam ist aber, dass lediglich die Testosteronproduktion in den Testes, nicht aber die der Nebennieren beeinflusst wird. Diese in den Nebennieren produzierten Androgene (Dehydroepiandrosteron und Androstendion) machen etwa 10 % des Gesamtandrogenspiegels aus und können weiterhin zu einer Stimulation der PK-Zellen führen.

Eine Therapie mit Antiandrogenen allein mag unter dem Aspekt der zirkulierenden Restandrogene als Alternative erscheinen. Es hat sich aber gezeigt, dass die PK-Zellen sehr schnell in der Lage sind zu mutieren und dann die verabreichten Antiandrogene als Agonisten an ihren Rezeptoren erkennen, was wiederum zum Tumorwachstum führt.[24-26]

Eine neue Form der Hormontherapie ist die vollständige Blockade von CYP17, dem Schlüsselenzym in der Androgenbiosynthese. Hierdurch ist es möglich, sowohl die testikuläre als auch die adrenale Androgenbiosynthese zu unterdrücken, womit schließlich eine vollständige Absenkung der Testosteronplasmalevel erreicht werden kann. Das Proof of Principle konnte hierfür mit dem Antimykotikum Ketoconazol (KTZ, Abb. 4) erbracht werden,[27] welches off-label zur Therapie des PK genutzt wird. Über die CYP17-Hemmung kann unter der Verabreichung von KTZ eine gute Absenkung des Testosteronplasmaspielgels erreicht werden. Der Nachteil dieser Substanz liegt allerdings in seiner unselektiven Hemmung einer Reihe von anderen CYP Enzymen, was mit schweren Nebenwirkungen bei einer Langzeitanwendung verbunden ist. Hierbei ist vor allem die Beeinträchtigung der hepatischen CYPs zu nennen, wodurch schwerwiegende Leberfunktionsstörungen hervorgerufen werden. Auch hemmt KTZ in hohem Maße das Schlüsselenzym der Cortisolbiosynthese CYP11B1, weshalb es meist nur in Kombination mit einem Cortisolderivat verabreicht werden kann.

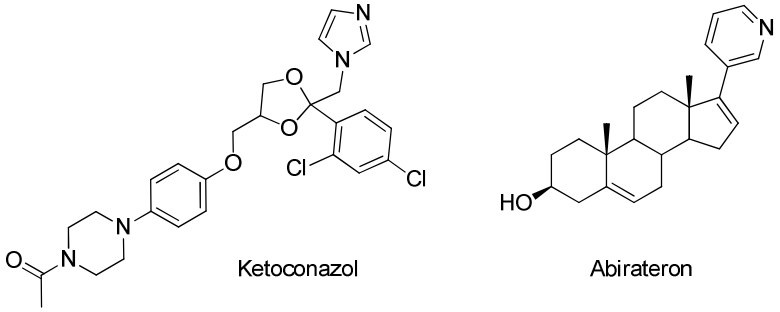

Abbildung 4: Die CYP17 Inhibitoren Ketoconazol und Abirateron

Die neueste Entwicklung auf diesem Gebiet ist die steroidale Verbindung Abirateron (ABT, Abb. 3), die als CYP17 Inhibitor in klinischen Studien derzeit die Phase III durchläuft und gute Aussichten auf eine Zulassung für die Prostatakrebstherapie hat.[28] Allerdings ist von Verbindungen, die auf steroidalen Grundgerüsten aufgebaut sind, bekannt, dass sie über Interaktionen mit Steroidrezeptoren zu unerwünschten Arzneimittelwirkungen führen können.

1.3 CYP11B1 als Drug Target

1.3.1 Cushing-Syndrom und cortisolabhängige Krankheiten

Das endogene Cushing-Syndrom ist eine Hormonstörung, die durch anhaltende Einwirkung hoher Cortisolspiegel verursacht wird. Es werden unterschiedliche Symptome ausgebildet, jedoch entwickeln die meisten Patienten das krankheitstypische Vollmondgesicht, Stammfettsucht, Osteoporose und Diabetes. Das Cushing-Syndrom ist verhältnismäßig selten und betrifft meist Erwachsene im Alter von 20 bis 50 Jahren. In etwa 80 % aller Fälle findet man eine hypophysäre Überproduktion von ACTH, deren Ursache meist ein ACTH produzierender Tumor der Hirnanhangsdrüse ist. In diesem Fall spricht man vom Morbus Cushing. Die ACTH unabhängige Form des Hypercortisolismus wird oft durch gut- oder bösartige Tumoren der Nebennieren hervorgerufen.[29]

Weiterhin ist ein Zusammenhang zwischen erhöhten Cortisolplasmaspiegeln und dem Metabolischen Syndrom sowie auch Diabetes bekannt.[30]

Im Jahr 2000 wurde durch Zhao *et al.* ein mutierter Androgenrezeptor in Prostatakarzinomzellen beschrieben, der sowohl durch Cortisol als auch durch seine inaktivierte Form Cortison aktiviert wird und damit eine Stimulation des Krebswachstums hervorruft.[31]

1.3.2 Therapie cortisolabhängiger Krankheiten

Beim Cushing-Syndrom ist die Behandlung der Wahl die Entfernung des Tumors oder eine angemessene Strahlentherapie. Trotzdem spricht mehr als ein Drittel aller Patienten nicht auf diese Therapieform an und benötigt weiterhin dauerhafte medikamentöse Behandlung. Bislang sind nur wenige Substanzen für die Therapie des Hypercortisolismus zugelassen. Sie werden abhängig von ihrem Wirkmechanismus in die folgenden drei Gruppen eingeteilt: Zum einen in Wirkstoffe, die direkt die ACTH Freisetzung aus der Hirnanhangdrüse beeinflussen. Sie werden auch als Neuromodulatoren bezeichnet und sind nur für die Therapie der ACTH abhängigen Krankheitsformen einsetzbar. Die zweite Klasse stellt die Inhibitoren der Cortisolbiosynthese dar. Sie blockieren die Cortisolbildung direkt in den Nebennieren und können somit bei allen Formen des Hypercortisolismus eingesetzt werden. Zuletzt befinden sich noch Glucocorticoidrezeptor-Blocker im Einsatz, die die Aktivierung der Rezeptoren durch das zirkulierende Cortisol verhindern sollen. Jedoch zeigen Studien mit dem Glucocorticoidrezeptor-Antagonist Mifepriston, dass seine Langzeitanwendung eine massive Cortisolausschüttung bewirkt, die von der Unterdrückung des natürlichen Feedback-Mechanismus verursacht wird. Daher erscheint eine Therapie mit Substanzen, die direkt die Bildung der Glucocorticoide unterdrücken, sprich CYP11B1-Inhibitoren, als am besten geeignete Option für alle

Arten des Cushing Syndroms. Hierzu sind bereits sieben Substanzen in klinischem Einsatz: Trilostane, Aminogluthetimid, Metyrapon, Ketoconazol, Fluconazol, Mitotan und Etomidat. Diese Wirkstoffe liefern zwar gute Ergebnisse, was den Rückgang der Cushing-Syndroms betrifft, allerdings zeigen alle auch schwere Nebenwirkungen, die durch ihre unselektive Hemmung anderer CYP Enzyme und Hydroxysteroiddehydrogenasen (HSDs) hervorgerufen werden.

Eine interessante, weitere Anwendung der CYP11B1 Inhibitoren stellt die Therapie des Metabolischen Syndroms oder auch Typ 2 Diabetes dar, da hier ebenfalls erhöhte Cortisolspiegel eine zentrale Rolle spielen. Eine kürzlich veröffentlichte Phase II Studie an Patienten mit Typ 2 Diabetes und weiteren Krankheitsbildern des metabolischen Syndroms mit dem 2*S*,4*R*-Enantiomer von Ketoconazol (DIO-902) zeigt vielversprechende Ergebnisse. Es konnte gezeigt werden, dass die Verabreichung von Ketokonazol eine Reduktion der HbA1c- und Cholesterol-Werte zur Folge hatte und auch zur Gewichtsreduktion sowie Blutdrucksenkung führte.[32] Leider musste die darauf aufbauende Langzeitstudie aufgrund der Ketoconazol-typischen Toxizität abgebrochen werden.

2 Ziel der Arbeit

2.1 Design und Synthese neuer nichtsteroidaler CYP17 Inhibitoren zur Therapie des Prostatakarzinoms.

Endogen synthetisierte Androgene sind ein maßgeblicher Triggerfaktor des hormonabhängigen Prostatakarzinoms. Daher ist ein neuer Angriffspunkt zur Therapie dieser Erkrankung die gezielte Hemmung des Schlüsselenzyms der Androgenbiosynthese, CYP17. Das Proof of Principle wurde bereits mit den klinisch angewandten Substanzen Ketoconazol und Abirateron erbracht.
Da Ketoconazol erhebliche Nebenwirkungen durch unselektive Hemmung weiterer CYP Enzyme verursacht und Abirateron auf einer steroidalen Grundstruktur beruht, die nicht vorhersehbare Interaktionen mit Steroidrezeptoren zeigen könnte, hat sich unser Arbeitskreis auf die Synthese nichtsteroidaler CYP17 Hemmstoffe spezialisiert. Durch Kombination eines liganden – und strukturbasierten Ansatzes und folgender gezielter Analyse der Struktur-Aktivitäts-Beziehungen sollen neue Hemmstoffe entwickelt und bezüglich ihrer Aktivität und Selektivität verbessert werden.

2.2 Entwicklung der ersten selektiven Hemmstoffe der 11β-Steroid Hydroxylase (CYP11B1) zur Therapie cortisol-abhängiger Krankheiten

Erhöhte Cortisolspiegel sind mit einer Reihe von Krankheitsbildern verbunden. Das bekannteste ist das Cushing-Syndrom, daneben zeigen aber auch Patienten mit Typ 2 Diabetes und metabolischem Syndrom klinisch erhöhte Cortisolplasmapiegel. Zur Therapie des Cushing-Syndroms werden bereits eine Reihe unselektiver Inhibitoren der Cortisol Synthase, wie Metyrapon, Etomidat oder Ketoconazol eingesetzt, ihre Anwendung ist aber aufgrund der gegebenen Unselektivität mit einer Reihe schwerer Nebenwirkungen verbunden. Daher sollen in einem neuen Projekt unserer Arbeitgruppe selektive nichtsteroidale CYP11B1-Hemmstoffe entwickelt und synthetisiert werden.

3 Ergebnisse

3.1 Novel CYP17 inhibitors: Synthesis, biological evaluation, structure-activity relationships and modelling of methoxy- and hydroxy-substituted methyleneimidazolyl biphenyls

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Paper I

Abstract:

Recently, the steroidal CYP17 inhibitor Abiraterone entered phase II clinical trial for the treatment of androgen dependent prostate cancer. As 17α-hydroxylase-17,20-lyase (CYP17) catalyzes the last step in androgen biosynthesis, inhibition of this target should affect not only testicular but also adrenal androgen formation. Therefore, CYP17 inhibitors should be advantageous over existing therapies, for example with GnRH analogues. However, steroidal drugs are known for side-effects which are due to affinities for steroid receptors. Therefore, we decided to synthesize non-steroidal compounds mimicking the natural CYP17 substrates pregnenolone and progesterone. The synthesis and biological evaluation of a series of 15 novel and highly active non-steroidal CYP17 inhibitors are reported. The compounds were prepared via Suzuki-cross-coupling, Grignard reaction and CDI-assisted S_Nt-reaction with imidazole and their inhibitory activity was examined with recombinant human CYP17 expressed in E. coli. Promising compounds were further tested for their selectivity against the hepatic enzyme CYP3A4 and the glucocorticoid forming enzyme CYP11B1. All compounds turned out to be potent

CYP17 inhibitors. The most active compounds **7** and **8** were much more active than Ketoconazole showing activity comparable to Abiraterone (IC_{50} values of 90 and 52 nM vs 72 nM). Most compounds also showed higher selectivities than Ketoconazole, but turned out to be less selective than Abiraterone. Docking studies using our CYP17 protein model were performed with selected compounds to study the interactions between the inhibitors and the amino acid residues of the active site.

Introduction:

Prostate Cancer (PC) is the most prevalent cancer in men in the US and Europe.[1] Since about 80% of patients with PC have androgen-dependent disease and respond to hormonal ablation, the presently used treatment is surgical castration (orchidectomy) or its medical equivalent, the application of gonadotropin-releasing hormone (GnRH) analogs to suppress testicular androgen biosynthesis.[2] However, only testicular production of androgens is affected by these strategies, the adrenal formation of androstendione is not and even after 3 months treatment with a GnRH agonist, prostate levels of testosterone and dihydrotestosterone are still about 25 and 10%, respectively.[3] Therefore, there is frequently combination with antiandrogens to counteract the stimulatory effect of androgens on the androgen receptor.4 However, it is speculated that due to mutations in the androgen receptor, anti-androgens might be recognized as agonists,[5,6] making this so called "combined androgen blockade" therapy not suitable for all patients.

A promising novel target for the treatment of prostate cancer is 17α-hydroxylase-17,20-lyase (CYP17), the cytochrome b_5 modulated key enzyme[7] for the biosynthesis of androgens, catalysing the 17α-hydroxylation of pregnenolone and progesterone and the subsequent cleavage of the C 20,21-acetyl group to yield the corresponding androgens dehydroepiandrosterone and androstendione (Fig. 1).[8]

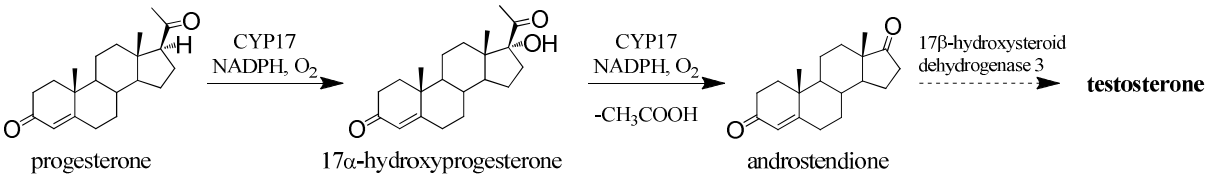
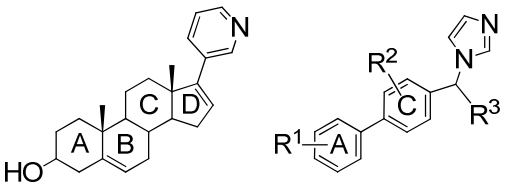


Fig. 1. The role of CYP 17 in androgen biosynthesis

Proof of principle was achieved by the antimycotic Ketoconazole, which clinically turned out to be a good adjuvant therapeutic capable of reducing testosterone levels through unspecific inhibition of CYP17.[9,10] Nevertheless, the side-effects it showed were the reason why it was not generally

accepted.[4] These drawbacks motivated us and others to look for more active and selective CYP17 inhibitors (for reviews see ref.: 11-16). Recently, the steroidal CYP17 inhibitor Abiraterone (Fig.2) passed phase II clinical trials showing high activity in post-docetaxel castration refractory PC patients and seems to have no dose-limiting toxicity.[17]



Abiraterone

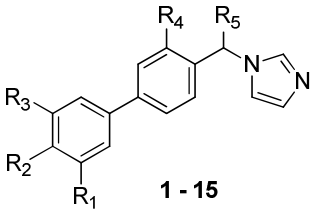
Fig. 2. Schematic presentation of Abiraterone and the scaffold of our biaryl inhibitors.

In previous works, we described novel in vitro and in vivo active steroidal[18-22] and non-steroidal[22-35] CYP17 inhibitors. Important for the mode of action of these compounds is a nitrogen-bearing heterocycle which is capable of complexing the heme iron of the enzyme. Very recently we demonstrated that some imidazole-methyl substituted biphenyls designed as AC-ring steroidal mimetics are good CYP17 inhibitors showing moderate selectivity toward other CYP enzymes.[32]

In this work, in order to further increase the inhibitory activity of these compounds, different substitution patterns of methoxy- and hydroxy-groups in the A-ring and further selected functional groups in the A- and C-ring were examined. Methyl and ethyl substituents were introduced into the methylene bridge between the biphenyl moiety and the imidazole ring, as we found in previous works,[30] that these substituents increase inhibitory potency.

In the following we report about the synthesis of compounds **1** – **15** (Table 1) and the evaluation of their inhibitory activities toward CYP17 and, for reasons of selectivity, the hepatic enzyme CYP3A4 and the glucocorticoid forming enzyme CYP11B1. Furthermore molecular modelling studies were performed with selected compounds.

Table 1
Inhibition of CYP17 by compounds **1**-**15**



1 - 15

Comp.	Structures					CYP17		
						% Inhibition [a]		IC_{50} [b]
	R^1	R^2	R^3	R^4	R^5	0.2 µM	2 µM	[nM]
Ref1 [c]	H	OH	H	H	H	1	32	
1	H	OMe	H	H	Me	9	40	
2	OMe	H	H	H	Me	8	59	
3	H	OH	H	H	Et	27	80	231
4	OH	H	H	H	Et	55	89	164
5	OH	OH	H	H	Et	56	91	152
6	OH	H	OH	H	Et	48	90	195
7	H	OH	H	F	Et	64	94	90
8	OH	OH	H	F	Et	69	95	52
9	H	OMe	H	H	Et	2	36	
10	OMe	H	H	H	Et	31	83	188
11	OMe	OMe	H	H	Et	9	58	
12	H	OEt	H	H	Et	3	25	
13	Me	OH	Me	H	Et	28	78	379
14	Me	OH	H	H	Et	45	89	261
15	Cl	OH	H	H	Et	44	89	217
KTZ [d]								2780
ABT [d]								72

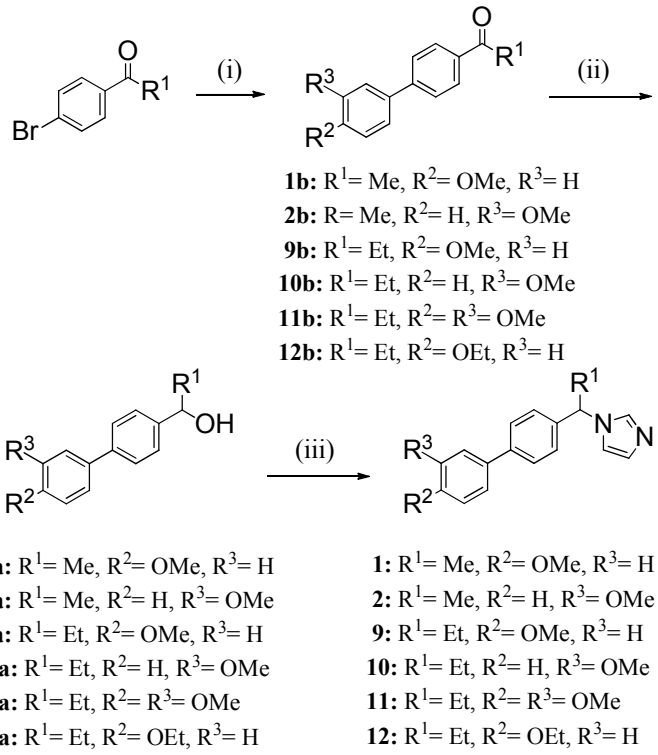
[a] Concentration of progesterone (substrate): 25 µM; standard deviations were within < ±5 %
[b] Concentration of inhibitors required to give 50 % inhibition. The given values are mean values of at least three experiments. The deviations were within ±10 %
[c] From Ref [32]; the IC_{50} value of 0.31 µM cited therein was obtained using a different source of the enzyme: human testicular microsoma
[d] **KTZ**: ketoconazole, **ABT**: abiraterone

Results

Chemistry

The syntheses of compounds **1** – **15** are shown in Schemes 1 and 2. Regarding the functional groups attached to the A-ring, the substances can be divided in alkoxy- and hydroxy-substituted compounds. In case of the methoxy- and ethoxy-substituted compounds **1**, **2** and **9** – **12** (Scheme 1), commercially available substituted phenylboronic acids were coupled to bromopropiophenone in a Suzuki reaction[36]

yielding the ketones **9b** – **12b**, and were subsequently reduced with $NaBH_4$ to the corresponding secondary alcohols **1a**, **2a** and **9a** – **12a**. The ketones **1b** and **2b** were commercially available.



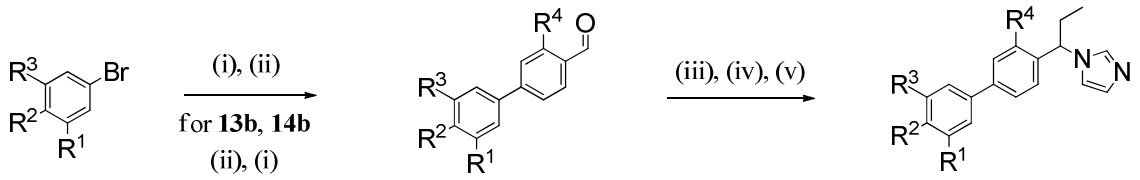
1b: R^1= Me, R^2= OMe, R^3= H
2b: R= Me, R^2= H, R^3= OMe
9b: R^1= Et, R^2= OMe, R^3= H
10b: R^1= Et, R^2= H, R^3= OMe
11b: R^1= Et, R^2= R^3= OMe
12b: R^1= Et, R^2= OEt, R^3= H

1a: R^1= Me, R^2= OMe, R^3= H
2a: R^1= Me, R^2= H, R^3= OMe
9a: R^1= Et, R^2= OMe, R^3= H
10a: R^1= Et, R^2= H, R^3= OMe
11a: R^1= Et, R^2= R^3= OMe
12a: R^1= Et, R^2= OEt, R^3= H

1: R^1= Me, R^2= OMe, R^3= H
2: R^1= Me, R^2= H, R^3= OMe
9: R^1= Et, R^2= OMe, R^3= H
10: R^1= Et, R^2= H, R^3= OMe
11: R^1= Et, R^2= R^3= OMe
12: R^1= Et, R^2= OEt, R^3= H

Scheme 1. Reagents and conditions: (i) $R^2R^3C_6H_3B(OH)_2$, Na_2CO_3, $Pd(PPh_3)_4$, toluene/MeOH/H_2O, 70 °C, 5 h; (ii) $NaBH_4$, MeOH, reflux, 2 h; (iii) CDI, NMP, 170 °C, 7 h

The hydroxy-substituted compounds **3** – **8** and **13** - **15** (Scheme 2) were also prepared via Suzuki cross-coupling reaction using phenylboronic acids carrying the carbonyl function and substituted bromophenols to yield the ketones **3b** – **8b**, **13c** – **14c** and **15b**. Most of the bromophenols (**3d**, **4d**, **13d** – **15d**) were commercially available. Demethylation of bromoveratrole and 1-bromo-3,5-dimethoxybenzene was achieved with BBr_3 (Method A) to yield **5d** and **6d**. The introduction of the ethyl substituent at the methylene bridge between the biphenylic core and the imidazole was performed by Grignard reaction, yielding the desired secondary alcohols **3a** – **8a** and **13a** – **15a**.



3d: R^1= R^3= H, R^2= OH,
4d: R^1= OH, R^2= R^3= H
5d: R^1= R^2= OH, R^3= H
6d: R^1= R^3= OH, R^2= H
13d: R^1= R^3= Me, R^2= OH
14d: R^1= Me, R^2= OH, R^3= H
15d: R^1= Cl, R^2= OH, R^3= H

3b: R^1= R^3= R^4= H, R^2= TBDMSO,
4b: R^1= TBDMSO, R^2= R^3= R^4= H
5b: R^1= R^2= TBDMSO ,R^3= R^4= H
6b: R^1= R^3= TBDMSO, R^2= R^4= H
7b: R^1= R^3= H, R^2= TBDMSO, R^4= F
8b: R^1= R^2= TBDMSO,R^3= H, R^4= F
13b: R^1= R^3= Me, R^2=TBDMSO, R^4= H
14b: R^1= Me, R^2=TBDMSO, R^3= R^4= H
15b: R^1= Cl, R^2=TBDMSO, R^3= R^4= H

3: R^1= R^3= R^4 = H, R^2= OH,
4: R^1= OH, R^2 = R^3= R^4= H
5: R^1= R^2= OH ,R^3= R^4= H
6: R^1= R^3= OH, R^2= R^4= H
7: R^1= R^3 = H, R^2= OH, R^4= F
8: R^1= R^2= OH,R^3= H, R^4= F
13: R^1= R^3= Me, R^2= OH, R^4= H
14: R^1= Me, R^2= OH, R^3= R^4= H
15: R^1= Cl, R^2= OH, R^3= R^4= H

Scheme 2. Reagents and conditions: (i) TBDMSCl, CH_2Cl_2, imidazole, rt, 12 h; (ii) phenyl boronic acid, Na_2CO_3, $Pd(PPh_3)_4$, toluene/MeOH/H_2O, 70 °C, 5 h; (iii) EtMgBr, THF, rt, 5 h; (iv) CDI, NMP, 170 °C, 7 h; (v) TBAF, THF, rt, 12 h.

All these secondary alcohols **1a** – **15a** were subjected to a S_N1 reaction with N,N'-carbonyldiimidazole (CDI) to give the imidazole-substituted compounds **1** – **15** as racemates [37].

The phenolic OH-groups had to be silyl-protected before the Grignard reaction. In most cases this synthetic step was performed before the Suzuki-reaction, as the chromatographic purification after the cross-coupling was easier with the protected compounds. For the final deprotection of the silylated compounds, TBAF was used as standard reagent.

Biological Results

Inhibition of human CYP17 was determined by performing our previously described assay [33] at inhibitor concentrations of 0.2 and 2 µM. In case of the most potent inhibitors IC_{50} values were determined. As source of human CYP17, our *E. coli* system[38] stably expressing human CYP17 and NADPH-P450 reductase was used. After homogenisation the 50,000 g sediment was incubated with progesterone (25 µM) as substrate and NADPH as previously described[27]. Separation of the product was accomplished by HPLC using UV-detection.

The inhibitory activities of compounds **1** – **15** and the reference compounds Abiraterone, Ketoconazole and **Ref1**[32] toward human CYP17 are shown in Table 1. The compounds can be divided in two classes according to the substituents at the A-ring, namely hydroxy and methoxy derivatives and also in two classes regarding substitution at the methylene bridge, in methyl and ethyl bearing compounds. All compounds were tested as racemates and showed inhibitory activity. The methoxy substituted compounds turned out to be weaker inhibitors compared to the corresponding hydroxy analogues. An exception to this is the 3-OCH_3 compound **10** which showed a similar high activity than the corresponding hydroxy compound **4** (IC_{50} = 188 nM and 164 nM). Comparing **10** which bears an

ethyl group at the methylene bridge with the correspondingly substituted methyl compound 2 clearly showed the significance of the alkyl substitution in this position, which we already have seen with similar compounds.[30,32,34,39] Furthermore it is striking that methoxy substitution in para position (R2) is not tolerated as the corresponding compounds **1**, **9** and **11** showed little activites.

The hydroxy compounds **3** - **8** and **13** – **15** showed very high inhibition (IC_{50} = 379 - 52 nM) with up to fifty fold better IC_{50} values than the reference Ketoconazole (IC_{50} = 2780 nM). They also turned out to be much more active than Ref1 which is certainly due to the ethyl group at the methylene linker. The additional introduction of substituents in compound **3** led to interesting findings. In case of the A-ring, substitution with another OH group is favourable (**5**), while CH_3 or Cl groups did not increase activity (**13** – **15**). Introduction of a F in the C-ring, however, increased the potency and led to the most active inhibitors of this study, compounds **7** and **8** (IC_{50} = 90 and 52 nM), the latter even exceeding Abiraterone (IC_{50} = 72 nM).

Regarding selectivity against other CYP enzymes, most compounds were tested for inhibition of the hepatic enzyme CYP3A4 at concentrations of 1 and 10 µM. This enzyme is involved in the metabolism of 50 % of the drugs. Its inhibition leads to drug-drug interactions by prolonging the half-lives of other coadministered drugs. All of the tested compounds showed inhibition of CYP3A4. The ones carrying only one hydroxy substituent at the A-ring (**Ref1** and compounds **3**, **4**, **7**; IC_{50} > 100 nM, data shown in supplementary material) were less potent than Ketoconazole (IC_{50} = 72 nM), which means they are more selective than the reference. The methoxy-substituted compounds **9**, **10** and **11** were even more selective (IC_{50} > 200 nM), however, not reaching Abiraterone (IC_{50} = 2704 nM)

Additionally, the most promising compounds were tested for inhibition of the steroidogenic CYP enzyme CYP11B1, which is catalyzing the key step in glucocorticoid biosynthesis. For the assay,[40] V79MZh11B1 cells expressing human CYP11B1 were used and the compounds were tested at concentrations 0.2 and 2 µM. While compounds **3**, **4**, **6**, **7**, **14** and **15** showed high inhibition of this enzyme at both concentrations (>89% at 0.2 µM and >95% at 2.0 µM, data shown in supplementary material), compounds 5, 8 and 13 exhibited less inhibitory activity toward this enzyme (38 – 51% at 0.2 µM). Interestingly, in contrast to the inhibition of CYP3A4, here the compounds with two vicinal OH-groups showed a very high selectivity and were better than Ketoconazole (61% at 0.2 µM). However, they did not reach Abiraterone (IC_{50} = 1608 nM). Because of this lack of selectivity, the compounds were not further tested for inhibition of other steroidogenic CYP enzymes like CYP11B2 and CYP19.

Molecular Modelling Studies

Both enantiomers, if existing, of selected energy minimized compounds (**3** – **11**, **13** – **15**) were docked into our CYP17 model by means of the GOLD v3.2 software[41] running Linux CentOS 5.1 on Intel(R) P4 CPU 3.00GHz. A slightly modified GOLDSCORE function with goldscore.P450_pdb.parameters,

for better evaluation of hydrophobic interactions, was used. The experimentally proven complexation of the heme iron with sp2 hybridized nitrogen[22] was considered in these studies by applying a distance constraint between those two atoms.

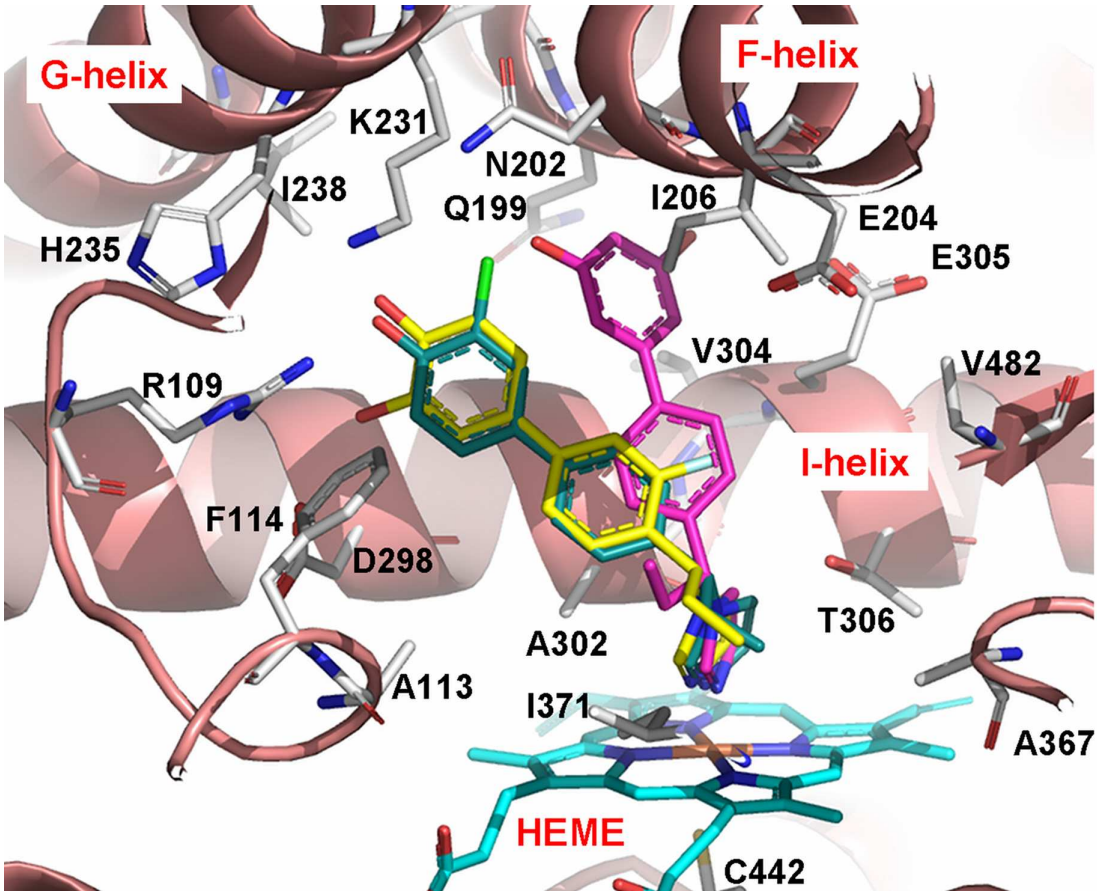


Figure 3. Presentation of the two found binding modes **BM1** (compounds **8** yellow and **15** green-blue) and **BM2** (compound **6** magenta). Heme (cyan), interacting residues and ribbon rendered tertiary structure of the active site are shown. Figure was generated with Pymol (http://www.pymol.org).

For both enantiomers of almost all docked compounds the statistical significant binding mode was **BM1** – the alternative mode **BM2** was less prevalent (Figure 3). These two binding modes could be previously identified by us for biphenylic CYP17 inhibitors[26,32]. In **BM1** the biaryl skeleton is oriented parallel to the I-helix pointing the A-ring next to a polar area. Hydrophobic and π-π interactions between the conjugated biphenyl core and Phe114 as well as apolar amino acid residues (Gly301, Ala302, Glu305, Ala367 and Ile371) were observed for all compounds.

The ethyl group at the methylene spacer has an important stabilizing role, as already described by us.[34] It anchors in a tiny hydrophobic pocket next to the heme, delimitated in its extend by Val366 and Ile371.

The analysis of the docking results was focused on the interaction of the A-ring with the corresponding amino acid residues, since most of the variations on the compounds presented in this

article are located here. Regarding the substituents in para position**,** it can be observed that a hydroxy group is involved in a H-bond net with Arg109, Lys231, His235 and Asp298. In contrast to this, for a methoxy group there is steric hindrance due to His235, Arg109 and the proximal I-helix residues. Depending on the different kind of substituents at the meta position, the R^1 group is oriented toward the F-/G-helix (Asn202, Lys231), if R^1 is MeO or Me or toward Asp298 for OH. The latter group forms a strong H-bond with the carboxylate (r = 1.9 Å). In case of the Cl-substituent, it was not possible to determine an unequivocal orientation, because of the bivalent interaction character of this halogen. Looking at the exact orientation of the MeO group, it becomes apparent that it is oriented toward a small hydrophobic pocket, delimitated in its extent by Ile238, Lys231, Ile206, Asn202 and Gln199. Its methyl group is placed at about 3.5 – 5 Å from the hydrophobic interaction partners of the mentioned amino acids, while its oxygen showed a reasonable H-bond with Lys231.
Striking for compounds **7** and **8** is the influence of the fluorine in the C-ring which reduces its electrostatic potential. Thus interactions with the backbone atoms of Gly301, Ala302 and Val304 are formed as we already have observed with other inhibitors of this class.[32]

Discussion and Conclusion

CYP17 is the pivotal enzyme in the biosynthesis of androgens, which are known to promote prostatic tumor growth. GnRH analogs have been shown to be useful in the treatment of prostate cancer by reducing the androgen formation. As outlined above inhibitors of CYP17 could be more efficient therapeutics as they should not only affect testicular androgen formation but also adrenal biosynthesis.

The present work describes a series of new imidazole substituted biphenyls. As they are very potent inhibitors of the target enzyme, our search for new substitution patterns increasing the activity of known biphenylic compounds[30-35,39] (e.g. **Ref1**) was successful. Regarding the structure-activity relationships obtained in this study, the importance of an ethyl substituent at the methylene bridge was demonstrated and could be explained in the modelling study. With respect to A-ring substitution a strong enhancement of the inhibitory activity was achieved by introduction of hydroxy groups, especially by two vicinal groups in R^1 and R^2. A very important role plays the fluorine substituent in the C-ring leading to the most active compounds **7** and **8**.

All synthesized compounds are much more potent than the reference Ketoconazole and two compounds reached the inhibitory activity of Abiraterone, which is under clinical investigation. Regarding selectivity toward the hepatic CYP3A4 and the glucocorticoid forming CYP11B1, most of the compounds turned out to be superior to Ketoconazole. However, none of the novel compounds reached the selectivity of the steroidal inhibitor Abiraterone. Therefore further structural optimization has to be performed to overcome this weakness. In our opinion this should be worthwhile as nonsteroidal inhibitors should not interfere with steroid hormone receptors as steroidal drugs do. These

studies are presently being performed. The structurally modified compounds will not only be tested for CYP3A4 and CYP11B1 selectivity but also for inhibition of other important hepatic CYP enzymes and steroidogenic CYPs like CYP11B2 and CYP19. As the enantioselectivity of the enzyme is an interesting aspect, the enantiomeric separation of the structural optimized compounds will be performed and the enantiomers will be evaluated.

Experimental Section

Biology Section

CYP17 preparation and assay

As source of human CYP17, our *E. coli* system[38] coexpressing human CYP17 and NADPH-P450 reductase was used and the assay was performed as previously described[27] using unlabeled progesterone as substrate and applying HPLC with UV-detection for separation.

Inhibition of hepatic enzyme CYP3A4

The recombinantly expressed enzyme from baculovirus-infected insect microsomes (Supersomes) was used and the manufacturer's instructions (www.gentest.com) were followed.

Inhibition of CYP11B1

V79MZh11B1 cells expressing CYP11B1 were used and our assay procedure with [4-^{14}C]-11-deoxycorticosterone was applied.[42]

Chemistry Section

General

Melting points were determined on a Mettler FP1 melting point apparatus and are uncorrected. IR spectra were recorded neat on a Bruker Vector 33FT-infrared spectrometer. ^{1}H-NMR spectra were measured on a Bruker DRX-500 (500 MHz). Chemical shifts are given in parts per million (ppm), and TMS was used as an internal standard for spectra obtained in $CDCl_3$. All coupling constants (*J*) are given in Hz. ESI (electrospray ionization) mass spectra were determined on a TSQ quantum (Thermo Electron Corporation) instrument. Elemental analyses were performed at the Department of Instrumental Analysis and Bioanalysis, Saarland University. Column chromatography was performed using silica-gel 60 (50-200 µm), and reaction progress was determined by TLC analysis on Alugram® SIL G/UV_{254} (Macherey-Nagel). Boronic acids and bromoaryls used as starting materials were obtained commercially (CombiBlocks, Chempur, Aldrich, Acros).

Method A: Boron Tribromide Cleavage of Phenolic Methyl Ethers

To a solution of the corresponding methyl ether in dichloromethane (300 mL) cooled in an ice bath was added BBr_3 (1.5 eq per MeO) dropwise. After 2 h, the mixture was allowed to warm to room temperature and then stirred overnight. Methanol (10 mL) was added dropwise to terminate the reaction, the mixture was poured into water and stirred for another 2 h. Then saturated $NaHCO_3$-solution (100 mL) was added, and the mixture was extracted with dichloromethane. The extracts were

washed with saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4 and concentrated. The resulting crude product was subjected to flash chromatography using silica gel.

4-Bromo-benzene-1,2-diol (5d)

Synthesised according to Method A using 4-bromo-1,2-dimethoxybenzene (8.00 g, 36.9 mmol) and BBr_3 (25.00 g, 100 mmol); pale oil; yield: 6.57 g (94 %); R_f = 0.38 (hexane / EtOAc, 9:1); δ_H ($CDCl_3$, 500 MHz): 5.18 (s, 1H), 5.33 (s, 1H), 6.74 (d, J = 8.5 Hz, 1H), 6.93 (dd, J = 2.2, 8.5 Hz, 1H), 7.02 (d, J = 2.2 Hz, 1H); δ_C ($CDCl_3$, 125 MHz): 112.6, 116.7, 118.7, 124.0, 142.7, 144.4; MS (ESI): m/z = 190 [M^++H].

5-Bromo-benzene-1,3-diol (6d)

Synthesised according to Method A using 1-bromo-3,5-dimethoxybenzene (8.00 g, 36.9 mmol) and BBr_3 (25.00 g, 100 mmol); pale oil; yield: 6.77 g (97 %). R_f = 0.38 (DCM / MeOH, 98:2); δ_H ($CDCl_3$, 500 MHz): 5.20 (s, 2H), 6.28 (t, J = 2.2 Hz, 1H), 6.67 (d, J = 2.2 Hz, 2H); MS (ESI): m/z = 190 [M^++H].

Method B: Protection of Phenols as TBDMS Ethers

To a solution of the corresponding phenol and imidazole (1.1 eq per OH) in dichloromethane, a solution of *tert*-butyldimethylsilyl chloride in dichloromethane was slowly added (1.1 eq per OH). After being stirred for 4 h at rt, the reaction mixture was poured into water, extracted with dichloromethane, washed with water and brine and dried over Na_2SO_4. Solvent removal under reduced pressure led to a pale oil, which was purified by chromatography on silica gel.

(4-Bromo-phenoxy)-tert-butyl-dimethyl-silane (3c)

Synthesised according to Method B using 4-bromophenol (**3d**) (3.00 g, 17.3 mmol); pale oil; yield: 4.80 g (94 %); R_f = 0.84 (hexane / EtOAc, 9:1); δ_H ($CDCl_3$, 500 MHz): 0.19 (s, 6H), 0.98 (s, 9H), 6.72 (d, J = 8.8 Hz, 2H), 7.32 (d, J = 8.8 Hz, 2H); δ_C ($CDCl_3$, 125 MHz): -4.5, 18.2, 25.6, 113.6, 121.9, 132.3, 154.8; MS (ESI): m/z = 288 [M^++H].

(3-Bromo-phenoxy)-tert-butyl-dimethyl-silane (4c)

Synthesised according to Method B using 3-bromophenol (**4d**) (5.00 g, 28 mmol); pale oil; yield: 8.21 g (quant.); R_f = 0.81 (hexane / EtOAc, 9:1); δ_H ($CDCl_3$, 500 MHz): 0.19 (s, 6H), 0.98 (s, 9H), 6.79 – 6.81 (m, 1H), 7.01 – 7.12 (m, 3H); MS (ESI): m/z = 288 [M^++H].

4-Bromo-1,2-bis-(tert-butyl-dimethyl-silanyloxy)-benzene (5c)

Synthesised according to Method B using **5d** (3.57 g, 18.9 mmol); pale oil; yield: 6.64 g (84 %); R_f = 0.59 (hexane); δ_H ($CDCl_3$, 500 MHz): 0.19 (s, 6H), 0.21 (s, 6H), 0.98 (s, 9H), 0.99 (s, 9H), 6.69 (d, J = 8.5 Hz, 1H), 6.92 (dd, J = 2.5, 8.5 Hz, 1H), 6.95 (d, J = 2.5 Hz, 1H); δ_C ($CDCl_3$, 125 MHz): -4.18, -4.14, 18.42, 18.44, 25.86, 25.89, 112.7, 122.2, 124.2, 124.3, 146.4, 147.9; MS (ESI): m/z = 418 [M^++H].

1-Bromo-3,5-bis-(tert-butyl-dimethyl-silanyloxy)-benzene (6c)

Synthesised according to Method B using **6d** (7.17 g, 38.0 mmol); pale oil; yield: 14.45 g (91 %); R_f = 0.73 (hexane); δ_H ($CDCl_3$, 500 MHz): 0.20 (s, 12H), 0.97 (s, 18H), 6.26 (t, *J* = 2.2 Hz, 1H), 6.63 (d, *J* = 2.2 Hz, 2H); MS (ESI): *m/z* = 418 [M^++H].

4'-(tert-Butyl-dimethyl-silanyloxy)-3',5'-dimethyl-biphenyl-4-carbaldehyde (13b)

Synthesised according to Method B using **13c** (0.92 g, 4.07 mmol); white solid; yield: 1.32 g (93 %); R_f = 0.33 (hexane / EtOAc, 10:1); δ_H ($CDCl_3$, 500 MHz): 0.25 (s, 6H), 1.08 (s, 9H), 2.31 (s, 6H), 7.28 (s, 2H), 7.70 (d, *J* = 8.3 Hz, 2H), 7.89 (d, *J* = 8.3 Hz, 2H), 10.01 (s, 1H, CHO); δ_C ($CDCl_3$, 125 MHz): -3.2, 17.6, 18.4, 25.8, 126.7, 127.4, 128.9, 129.8, 132.0, 134.2, 146.7, 152.6, 191.4; MS (ESI): *m/z* = 227 [M^++H].

4'-(tert-Butyl-dimethyl-silanyloxy)-3'-methyl-biphenyl-4-carbaldehyde (14b)

Synthesised according to Method B using **14c** (0.96 g, 4.07 mmol); white solid; yield: 1.40 g (95 %); R_f = 0.33 (hexane / EtOAc, 10:1); δ_H ($CDCl_3$, 500 MHz): 0.26 (s, 6H), 1.04 (s, 9H), 2.29 (s, 3H), 6.85 (d, *J* = 8.3 Hz, 1H), 7.36 (dd, *J* = 2.3, 8.3 Hz, 1H), 7.44 (s, 1H), 7.70 (d, *J* = 8.3 Hz, 2H), 7.91 (d, *J* = 8.4 Hz, 2H), 10.03 (s, 1H); δ_C ($CDCl_3$, 125 MHz): -4.4, 16.8, 25.5, 118.6, 125.4, 126.8, 130.0, 134.3, 146.8, 154.4, 191.7; MS (ESI): *m/z* = 227 [M^++H].

(4-Bromo-2-chloro-phenoxy)-tert-butyl-dimethyl-silane (15c)

Synthesised according to Method B using 4-bromo-2-chlorophenol (**15d**) (1.00 g, 4.82 mmol); white solid; yield: 1.40 g (91 %); R_f = 0.45 (hexane / EtOAc, 20:1); δ_H ($CDCl_3$, 500 MHz): 0.22 (s, 6H), 1.02 (s, 9H), 6.75 (d, *J* = 8.6 Hz, 1H), 7.23 (dd, *J* = 2.5, 8.6 Hz, 1H), 7.48 (d, *J* = 2.5 Hz, 1H); δ_C ($CDCl_3$, 125 MHz): -4.7, 25.3, 112.9, 121.6, 126.6, 130.2, 132.5, 150.7; MS (ESI): *m/z* = 322 [M^++H].

Method C: Suzuki-Coupling

To a solution of the corresponding bromobenzene derivative (1.0 eq) in toluene (7 mL / mmol), an aqueous Na_2CO_3 solution (2.0 M; 3.2 mL / mmol) and an ethanolic solution (3.2 mL / mmol) of the corresponding boronic acid (1.5-2.0 eq) were added. The mixture was deoxygenated under reduced pressure and flushed with nitrogen. After having repeated this cycle several times, $Pd(PPh_3)_4$ (4 mol%) was added, and the resulting suspension was heated under reflux for 8 h. After cooling, ethyl acetate (10 mL) and water (10 mL) were added. The organic phase was separated and the water phase was extracted with ethyl acetate (2 x 10 mL). The combined organic phases were washed with brine, dried over Na_2SO_4, filtered over a short plug of celite® and evaporated under reduced pressure. The compounds were purified by flash chromatography using silica gel.

4'-(tert-Butyl-dimethyl-silanyloxy)-biphenyl-4-carbaldehyde (3b)

Synthesised according to Method C using **3c** (4.80 g, 16.7 mmol) and 4-formylphenylboronic acid (5.01 g, 33.4 mmol); yellow solid; yield: 3.80 g (73 %); R_f = 0.65 (hexane / EtOAc, 9:1); δ_H ($CDCl_3$, 500 MHz): 0.25 (s, 6H), 1.02 (s, 9H), 6.94 (d, *J* = 8.8 Hz, 2H), 7.53 (d, *J* = 8.8 Hz, 2H), 7.71 (d, *J* = 8.2 Hz, 2H), 7.92 (d, *J* = 8.2 Hz, 2H), 10.03 (s, 1H); δ_C ($CDCl_3$, 125 MHz): -4.4, 18.2, 25.6, 120.6, 127.0, 127.6, 128.4, 130.2, 134.7, 146.8, 156.4, 191.8; MS (ESI): *m/z* = 297 [M^++H].

3'-(tert-Butyl-dimethyl-silanyloxy)-biphenyl-4-carbaldehyde (4b)

Synthesised according to Method C using **4c** (6.66 g, 22.9 mmol) and 4-formylphenylboronic acid (5.10 g, 34.5 mmol); yellow solid; yield: 3.70 g (52 %); R_f = 0.60 (hexane / EtOAc 9/1); the compound was directly used in the next step without further purification and analysis.

3',4'-Bis-(tert-butyl-dimethyl-silanyloxy)-biphenyl-4-carbaldehyde (5b)

Synthesised according to Method C using **5c** (5.30 g, 12.7 mmol) and 4-formylphenylboronic acid (2.80 g, 19.0 mmol); yellow solid; yield: 3.30 g (58 %); R_f = 0.40 (hexane / EtOAc, 9:1); the compound was directly used in the next step without further purification and analysis.

3',5'-Bis-(tert-butyl-dimethyl-silanyloxy)-biphenyl-4-carbaldehyde (6b)

Synthesised according to Method C using **6c** (11.0 g, 26.3 mmol) and 4-formylphenylboronic acid (6.00 g, 39.4 mmol); yellow solid; yield: 7.67 g (66 %); R_f = 0.59 (hexane / EtOAc, 9:1); the compound was directly used in the next step without further purification and analysis.

4'-(tert-Butyl-dimethyl-silanyloxy)-3-fluoro-biphenyl-4-carbaldehyde (7b)

Synthesised according to Method C using **3c** (3.50 g, 11.9 mmol) and 3-fluor-4-formylphenylboronic acid (3.00 g, 17.9 mmol); yellow solid; yield: 3.80 g (73 %); R_f = 0.47 (hexane / EtOAc, 9:1); the compound was directly used in the next step without further purification and analysis.

3',4'-Bis-(tert-butyl-dimethyl-silanyloxy)-3-fluoro-biphenyl-4-carbaldehyde (8b)

Synthesised according to Method C using **5c** (5.0 g, 12.0 mmol) and 3-fluor-4-formylphenylboronic acid (3.00 g, 17.9 mmol); yellow solid; yield: 3.70 g (67 %); R_f = 0.68 (hexane / EtOAc, 9:1); the compound was directly used in the next step without further purification and analysis.

1-(4'-Methoxy-biphenyl-4-yl)-propan-1-one (9b)

Synthesised according to Method C using 4'-bromopropiophenone (2.0 g, 9.4 mmol) and 4-methoxyphenylboronic acid (2.14 g, 14.1 mmol); yellow solid; yield: 2.12 g (94 %); R_f = 0.29 (DCM / hexane, 1:1); δ_H ($CDCl_3$, 500 MHz): 1.25 (t, J = 7.3 Hz, 3H), 3.02 (q, J = 7.3 Hz, 2H), 3.86 (s, 3H), 7.00 (d, J = 8.8 Hz, 2H), 7.57 (d, J = 8.8 Hz, 2H), 7.63 (d, J = 8.5 Hz, 2H), 8.01 (d, J = 8.5 Hz, 2H); δ_C ($CDCl_3$, 125 MHz): 8.3, 31.8, 55.4, 114.4, 126.6, 128.3, 128.6, 132.3, 135.0, 145.1, 159.9, 200.4; MS (ESI): m/z = 241 [M^++H].

1-(3'-Methoxy-biphenyl-4-yl)-propan-1-one (10b)

Synthesised according to Method C using 4'-bromopropiophenone (2.0 g, 9.4 mmol) and 3-methoxyphenylboronic acid (2.14 g, 14.1 mmol); orange solid; yield: 2.30 g (96 %); R_f = 0.29 (DCM / hexane, 1:1); δ_H ($CDCl_3$, 500 MHz): 1.25 (t, J = 7.3 Hz, 3H), 3.04 (q, J = 7.3 Hz, 2H), 3.88 (s, 3H), 6.95 (ddd, J = 0.6, 2.5, 8.2 Hz, 1H), 7.15 (t, J = 2.5 Hz, 1H), 7.20 – 7.22 (m, 1H), 7.39 (t, J = 8.2 Hz, 1H), 7.67 (d, J = 8.5 Hz, 2H), 8.03 (d, J = 8.5 Hz, 2H); δ_C ($CDCl_3$, 125 MHz): 8.3, 31.8, 55.3, 113.0, 113.5, 119.7, 127.3, 128.5, 130.0, 135.8, 141.4, 160.0, 200.4; MS (ESI): m/z = 241 [M^++H].

1-(3',4'-Dimethoxy-biphenyl-4-yl)-propan-1-one (11b)

Synthesised according to Method C using 4'-bromopropiophenone (2.00 g, 9.4 mmol) and 3,4-dimethoxyphenylboronic acid (2.57 g, 14.1 mmol); orange solid; yield: 2.41 g (95 %); R_f = 0.37

(DCM); δ_H ($CDCl_3$, 500 MHz): 1.25 (t, *J* = 7.3 Hz, 3H), 3.03 (q, *J* = 7.3 Hz, 2H), 3.94 (s, 3H), 3.97 (s, 3H), 6.97 (d, *J* = 8.2 Hz, 1H), 7.14 (d, *J* = 2.2 Hz, 1H), 7.20 (dd, *J* = 2.2, 8.2 Hz, 1H), 7.64 (d, *J* = 8.5 Hz, 2H), 8.02 (d, *J* = 8.5 Hz, 2H); MS (ESI): *m/z* = 271 [M^++H].

1-(4'-Ethoxy-biphenyl-4-yl)-propan-1-one (12b)

Synthesised according to Method C using 4'-bromopropiophenone (0.86 g, 4.0 mmol) and 4-ethoxyphenylboronic acid (1.00 g, 6.02 mmol); yellow solid; yield: 0.96 g (94 %); R_f = 0.32 (hexane / EtOAc, 9:1); δ_H ($CDCl_3$, 500 MHz): 1.25 (t, *J* = 7.3 Hz, 3H), 1.45 (t, *J* = 6.9 Hz, 3H), 3.03 (q, *J* = 7.3 Hz, 2H), 4.10 (q, *J* = 6.9 Hz, 2H), 6.68 (d, *J* = 8.8 Hz, 2H), 7.50 (d, *J* = 8.8 Hz, 2H), 7.64 (d, *J* = 8.2 Hz, 2H), 8.01 (d, *J* = 8.2 Hz, 2H); MS (ESI): *m/z* = 255 [M^++H].

4'-Hydroxy-3',5'-dimethyl-biphenyl-4-carbaldehyde (13c)

Synthesised according to Method C using 4-bromo-2,6-dimethylphenol (**13d**) (1.00 g, 4.97 mmol) and 4-formylphenyl boronic acid (0.89 g, 5.9 mmol); white solid; yield: 0.92 g (82 %); R_f = 0.30 (hexane / EtOAc, 5:1); δ_H ($CDCl_3$, 500 MHz): 2.33 (s, 6H), 4.81 (bs, 1H), 7.28 (s, 2H), 7.69 (d, *J* = 8.3 Hz, 2H), 7.90 (d, *J* = 8.3 Hz, 2H), 10.03 (s, 1H); δ_C ($CDCl_3$, 125 MHz): 15.8, 126.4, 126.8, 127.4, 130.0, 131.4, 134.3, 152.7, 191.7; MS (ESI): *m/z* = 227 [M^++H].

4'-Hydroxy-3'-methyl-biphenyl-4-carbaldehyde (14c)

Synthesised according to Method C using 4-bromo-2-methylphenol (**14d**) (1.00 g, 5.35 mmol) and 4-formylphenyl boronic acid (0.96 g, 6.4 mmol); white solid; yield: 0.96 g (85 %); R_f = 0.31 (hexane / EtOAc, 5:1); δ_H ($CDCl_3$, 500 MHz): 2.34 (s, 3H), 5.23 (bs, 1H), 6.89 (d, *J* = 8.3 Hz, 1H), 7.37 (dd, *J* = 2.3, 8.3 Hz, 1H), 7.43 (bs, 1H), 7.70 (d, *J* = 8.3 Hz, 2H), 7.91 (d, *J* = 8.4 Hz, 2H), 10.03 (s, 1H); δ_C ($CDCl_3$, 125 MHz): 15.7, 115.2, 124.3, 125.9, 126.8, 130.1, 131.9, 134.3, 146.8, 154.4, 191.9 (CHO); MS (ESI): *m/z* = 213 [M^++H].

4'-(tert-Butyl-dimethyl-silanyloxy)-3'-chloro-biphenyl-4-carbaldehyde (15b)

Synthesised according to Method C using **15c** (1.40 g, 4.35 mmol) and 4-formylphenyl boronic acid (0.78 g, 5.5 mmol); white solid; yield: 1.22 g (81 %); R_f = 0.33 (hexane / EtOAc, 10:1); δ_H ($CDCl_3$, 500 MHz): 0.27 (s, 6H), 1.06 (s, 9H), 6.98 (d, *J* = 8.4 Hz, 1H), 7.41 (dd, *J* = 2.3, 8.4 Hz, 1H), 7.65 (d, *J* = 2.3 Hz, 1H), 7.69 (d, *J* = 8.3 Hz, 2H), 7.93 (d, *J* = 8.4 Hz, 2H), 10.04 (s, 1H); δ_C ($CDCl_3$, 125 MHz): -4.4, 25.6, 121.1, 126.4, 127.1, 129.1, 130.3, 133.7, 135.1, 145.6, 152.1, 191.8; MS (ESI): *m/z* = 348 [M^++H].

Method D: Grignard-Reaction

Under exclusion of air and moisture, an EtMgBr-solution (1.0 M in THF, 1.2 eq) solution in THF was added dropwise to a solution of the aldehyde or ketone (1 eq) in THF (12 mL / mmol). The mixture was stirred overnight at rt. Then water (10 mL) and ethyl acetate (10 mL) were added. The organic phase was separated and washed with water and brine, dried over Na_2SO_4, and evaporated under reduced pressure. The crude products were purified by flash chromatography using silica gel.

1-[4'-(tert-Butyl-dimethyl-silanyloxy)-biphenyl-4-yl]-propan-1-ol (3a)

Synthesised according to Method D using **3b** (3.30 g, 10.6 mmol) and EtMgBr (1.0 M in THF, 12.7 mL, 12.7 mmol); white solid; yield: 1.89 g (52 %); R_f = 0.40 (hexane / EtOAc, 9:1); δ_H ($CDCl_3$, 500 MHz): 0.23 (s, 6H), 0.95 (t, *J* = 7.3 Hz, 3H), 1.00 (s, 9H), 1.76 – 1.89 (m, 2H), 4.64 (t, *J* = 6.6 Hz, 1H), 6.90 (d, *J* = 8.5 Hz, 2H), 7.38 (d, *J* = 8.5 Hz, 2H), 7.46 (d, *J* = 8.5 Hz, 2H), 7.54 (d, *J* = 8.5 Hz, 2H); MS (ESI): *m/z* = 344 [M^++H].

1-[3'-(tert-Butyl-dimethyl-silanyloxy)-biphenyl-4-yl]-propan-1-ol (4a)

Synthesised according to Method D using **4b** (3.70 g, 11.9 mmol) and EtMgBr (1.0 M, 14.3 mL, 14.3 mmol); white solid; yield: 2.02 g (45 %); R_f = 0.40 (hexane / EtOAc, 9:1); δ_H ($CDCl_3$, 500 MHz): 0.27 (s, 6H), 0.97 (t, *J* = 7.3 Hz, 1H), 1.05 (s, 9H), 1.76 – 1.91 (m, 2H), 2.15 – 2.38 (m, 1H), 4.65 (t, *J* = 6.6 Hz, 1H), 6.86 (m, 1H), 7.11 (m, 1H), 7.21 (d, J = 7.9 Hz, 1H), 7.31 (t, *J* = 7.9 Hz, 1H), 7.41 (d, *J* = 8.2 Hz, 2H), 7.57 (d, *J* = 8.2 Hz, 2H); δ_C ($CDCl_3$, 125 MHz): -4.4, 9.9, 21.2, 25.7, 29.2, 77.1, 118.8, 119.0, 120.1, 126.9, 127.1, 129.6, 139.6, 140.5, 142.2, 156.0; MS (ESI): *m/z* = 344 [M^++H].

1-[3',4'-Bis-(tert-butyl-dimethyl-silanyloxy)-biphenyl-4-yl]-propan-1-ol (5a)

Synthesised according to Method D using **5b** (6.09 g, 13.7 mmol) and EtMgBr (1.0 M, 16.4 mL, 16.4 mmol); white solid; yield: 1.55 g (25 %); R_f = 0.62 (hexane / EtOAc, 7:3); δ_H ($CDCl_3$, 500 MHz): 0.22 (s, 6H), 0.23 (s, 6H), 0.88 (t, *J* = 7.3 Hz, 3 H),1.00 (s, 9H), 1.01 (s, 9H), 1.68 – 1.81 (m, 2H), 2.51 (bs, 1H), 4.52 (t, *J* = 6.6 Hz, 1H), 6.86 (d, *J* = 8.2 Hz, 1H), 7.02 (dd, *J* = 2.2, 8.2 Hz, 1H), 7.08 (d, *J* = 2.2 Hz, 1H), 7.31 (d, *J* = 8.2 Hz, 2H), 7.45 (d, *J* = 8.2 Hz, 2H); δ_C ($CDCl_3$, 125 MHz): -4.08, -4.12, 10.1, 18.38, 18.39, 25.90, 25.92, 31.7, 75.5, 119.7, 119.9, 121.2, 126.3, 126.5, 134.3, 139.8, 143.1, 146.4, 146.9; MS (ESI): *m/z* = 474 [M^++H].

1-[3',5'-Bis-(tert-butyl-dimethyl-silanyloxy)-biphenyl-4-yl]-propan-1-ol (6a)

Synthesised according to Method D using **6b** (6.85 g, 15.5 mmol) and EtMgBr (1.0 M, 18.6 mL, 18.6 mmol); yellow solid; yield: 3.60 g (49 %); the compound was directly used in the next step without further purification and analysis.

1-[4'-(tert-Butyl-dimethyl-silanyloxy)-3-fluoro-biphenyl-4-yl]-propan-1-ol (7a)

Synthesised according to Method D using **7b** (3.01 g, 9.10 mmol) and EtMgBr (1.0 M, 10.9 mL, 10.9 mmol); white solid; yield: 1.30 g (40 %); R_f = 0.48 (hexane / EtOAc, 9:1); δ_H ($CDCl_3$, 500 MHz): 0.25 (s, 6H), 0.98 (t, *J* = 7.3 Hz, 3H), 1.03 (s, 9H), 1.79 – 1.89 (m, 2H), 2.33 (bs, 1H), 4.94 – 4.97 (m, 1H), 6.91 (d, *J* = 8.8 Hz, 2H), 7.20 (dd, *J* = 1.9, 12.0 Hz, 1H), 7.33 (dd, *J* = 1.9, 8.2 Hz, 1H), 7.44 – 7.46 (m, 3H); MS (ESI): *m/z* = 361 [M^++H].

1-[3',4'-Bis-(tert-butyl-dimethyl-silanyloxy)-3-fluoro-biphenyl-4-yl]-propan-1-ol (8a)

Synthesised according to Method D using **8b** (3.71 g, 8.00 mmol) and EtMgBr (1.0 M, 9.6 mL, 9.6 mmol); yellow solid; yield: 1.36 g (35 %); R_f = 0.34 (hexane / EtOAc, 9:1); the compound was directly used in the next step without further purification and analysis.

1-[4'-(tert-Butyl-dimethyl-silanyloxy)-3',5'-dimethyl-biphenyl-4-yl]-propan-1-ol (13a)

Synthesised according to Method D using **13b** (1.32 g, 3.88 mmol) and EtMgBr (1.0 M, 4.65 mL, 4.65 mmol) solution in THF. Yield: 1.24 g (86 %); R_f = 0.15 (hexane / EtOAc, 10:1); white solid; δ_H ($CDCl_3$, 500 MHz): 0.24 (s, 6H), 0.95 (t, *J* = 7.4 Hz, 3H), 1.08 (s, 9H), 1.77-1.88 (m, 2H), 1.93 (bs, 1H), 2.29 (s, 6H), 4.62 (t, *J* = 6.5 Hz, 1H), 7.23 (s, 2H), 7.37 (d, *J* = 8.3 Hz, 2H), 7.54 (d, *J* = 8.3 Hz, 2H); δ_C ($CDCl_3$, 125 MHz): -3.2, 9.9, 17.7, 25.8, 31.5, 75.5, 125.9, 126.4, 127.1, 128.6, 133.4, 140.0, 142.5, 151.6; MS (ESI): *m/z* = 227 [M^++H].

1-[4'-(tert-Butyl-dimethyl-silanyloxy)-3'-methyl-biphenyl-4-yl]-propan-1-ol (14a)

Synthesised according to Method D using **14b** (1.40 g, 4.29 mmol) with EtMgBr (1.0 M, 4.71 mL, 4.71 mmol) solution in THF; white solid; yield: 1.24 g (81 %); R_f = 0.15 (hexane / EtOAc, 10:1); the compound was directly used in the next step without further purification and analysis.

1-[4'-(tert-Butyl-dimethyl-silanyloxy)-3'-chloro-biphenyl-4-yl]-propan-1-ol (15a)

Synthesised according to Method D using **15b** (1.22 g, 3.52 mmol) with EtMgBr (1.0 M, 3.87 mL, 3.87 mmol) solution in THF; white solid; yield: 1.05 g (79 %); R_f = 0.18 (hexane / EtOAc, 10:1); the compound was directly used in the next step without further purification and analysis.

Method E: Reduction with $NaBH_4$

To an ice-cooled solution of the corresponding aldehyde or ketone (1.0 eq) in methanol (5 mL / mmol) was added $NaBH_4$ (2.0 eq). The resulting mixture was heated to reflux for 30 minutes. After cooling to ambient temperature, the solvent was distilled off under reduced pressure. Water (10 mL) was added, and the aqueous layer was extracted with ethyl acetate (3 x 10 mL). The combined organic phases were washed with brine, dried over $MgSO_4$ and evaporated under reduced pressure. The desired product was purified by chromatography using silica gel.

1-(4'-Methoxy-biphenyl-4-yl)-ethanol (1a)

Synthesised according to Method E using 1-(4'-methoxybiphenyl-4-yl)ethanone (**1b**) (0.23 g, 1.00 mmol) and $NaBH_4$ (0.15 g, 3.98 mmol); colourless solid; yield: 0.13 g (58 %); R_f = 0.16 (hexane / EtOAc, 10:1); the compound was directly used in the next step without further purification and analysis.

1-(3'-Methoxy-biphenyl-4-yl)-ethanol (2a)

Synthesised according to Method E using 1-(3'-methoxybiphenyl-4-yl)ethanone (**2b**) (0.62 g, 2.71 mmol) and $NaBH_4$ (0.19 g, 4.88 mmol); colourless solid; yield: 0.45 g (72 %); R_f = 0.17 (hexane / EtOAc, 10:1); IR (ATR) $\tilde{\upsilon}$ (cm^{-1}): 3388 (m), 1600 (m), 1481 (m), 1295 (m), 1214 (s), 1053 (m), 832 (s), 777 (s), 695 (m); δ_H ($CDCl_3$, 500 MHz): 1.54 (d, *J* = 6.4 Hz, 3H), 1.77 (bs, 1H), 3.87 (s, 3H), 4.96 (q, *J* = 6.4 Hz, 1H), 6.90 (dd, *J* = 2.2, 8.2 Hz, 1H), 7.12 (bt, *J* = 2.2 Hz, 1H), 7.17 - 7.19 (m, 1H), 7.36 (t, *J* = 7.9 Hz, 1H), 7.45 (d, *J* = 8.2 Hz, 2H), 7.58 (d, *J* = 8.2 Hz, 2H); δ_C ($CDCl_3$, 125 MHz): 25.1, 55.3, 70.1, 112.6, 112.8, 119.6, 125.8, 127.3, 129.7, 140.3, 142.4, 145.0, 159.9; MS (ESI): *m/z* = 211 [M^++H-H_2O].

1-(4'-Methoxy-biphenyl-4-yl)-propan-1-ol (9a)

Synthesised according to Method E using **9b** (2.12 g, 8.80 mmol) and $NaBH_4$ (0.64 g, 17 mmol); white solid; yield: 1.85 g (87 %); R_f = 0.37 (DCM); δ_H ($CDCl_3$, 500 MHz): 0.95 (t, *J* = 7.6 Hz, 3H), 1.77 – 1.89 (m, 3H), 3.85 (s, 3H), 4.64 (t, *J* = 6.6 Hz, 1H), 6.98 (d, *J* = 8.5 Hz, 2H), 7.39 (d, *J* = 8.5 Hz, 2H), 7.52 – 7.55 (m, 4H); δ_C ($CDCl_3$, 125 MHz): 10.2, 31.8, 55.3, 75.8, 114.2, 126.4, 126.7, 128.1, 133.4, 140.1, 143.0, 159.1; MS (ESI): *m/z* = 243 [M^++H].

1-(3'-Methoxy-biphenyl-4-yl)-propan-1-ol (10a)

Synthesised according to Method E using **10b** (2.30 g, 9.00 mmol) and $NaBH_4$ (0.76 g, 20 mmol); white solid; yield: 1.87 g (86 %); R_f = 0.37 (DCM); δ_H ($CDCl_3$, 500 MHz): 0.95 (t, *J* = 7.3 Hz, 3H), 1.78 – 1.90 (m, 3H), 3.88 (s, 3H), 4.65 (t, *J* = 6.4 Hz, 1H), 6.98 (ddd, *J* = 0.6, 2.5, 8.2 Hz, 1H), 7.17 (t, *J* = 2.5 Hz, 1H), 7.22 – 7.24 (m, 2H), 7.71 (d, *J* = 8.5 Hz, 2H), 8.06 (d, *J* = 8.5 Hz, 2H); δ_C ($CDCl_3$, 125 MHz): 10.3, 31.8, 55.3, 76.1, 113.0, 113.4, 119.7, 127.4, 128.2, 130.0, 135.8, 141.4, 159.9; MS (ESI): *m/z* = 243 [M^++H].

1-(3',4'-Dimethoxy-biphenyl-4-yl)-propan-1-ol (11a)

Synthesised according to Method E using **11b** (2.41 g, 8.90 mmol) and $NaBH_4$ (0.76 g, 20 mmol); yellow solid; yield: 2.03 g (84 %); R_f = 0.31 (DCM); δ_H ($CDCl_3$, 500 MHz): 0.99 (t, *J* = 7.3 Hz, 3H), 1.81- 1.90 (m, 3H), 3.82 (s, 3H), 3.84 (s, 3H), 4.59 (t, *J* = 6.6 Hz, 1H), 6.94 (d, *J* = 8.2 Hz, 1H), 7.07 (d, *J* = 2.2 Hz, 1H), 7.11 (dd, *J* = 2.2, 8.2 Hz, 1H), 7.30 (d, *J* = 8.2 Hz, 2H), 7.55 (d, *J* = 8.2 Hz, 2H); MS (ESI): *m/z* = 273 [M^++H].

1-(4'-Ethoxy-biphenyl-4-yl)-propan-1-ol (12a)

Synthesised according to Method E using **12b** (1.32 g, 5.20 mmol) and $NaBH_4$ (0.39 g, 10 mmol); white solid; yield: 1.14 g (86 %); R_f = 0.16 (hexane / EtOAc 9:1); δ_H ($CDCl_3$, 500 MHz): 0.95 (t, *J* = 7.3 Hz, 3H), 1.42 (t, *J* = 6.6 Hz, 3H), 2.22 - 2.29 (m, 2H), 4.07 (q, *J* = 6.6 Hz, 2H), 5.03 (t, *J* = 7.7 Hz, 1H), 6.97 (d, *J* = 8.8 Hz, 2H), 7.40 (d, *J* = 8.2 Hz, 2H), 7.53 – 7.56 (m, 4H); MS (ESI): *m/z* = 257 [M^++H].

Method F: CDI reaction (Compounds 1, 2, 9 – 12)

To a solution of the corresponding alcohol (1.0 eq) in *N*-Methyl-2-pyrrolidon (NMP) or acetonitrile (10 mL / mmol), CDI (5.0 eq) was added at rt. The solution was heated to reflux for 4 to 18 h. After cooling to ambient temperature, the reaction mixture was diluted with water (30 mL) and extracted with ethyl acetate (3 x 10 mL). The combined organic phases were washed with brine, dried over $MgSO_4$ and evaporated under reduced pressure. The crude product was purified via chromatography using silica gel.

1-[1-(4'-Methoxy-biphenyl-4-yl)-ethyl]-1H-imidazole (1)

Synthesised according to Method F using **1a** (0.20 g, 0.88 mmol) and CDI (0.28 g, 1.8 mmol) in acetonitrile; colourless solid; yield: 0.12 g (49 %); R_f = 0.15 (EtOAc); IR (ATR) $\tilde{\nu}$ (cm^{-1}): 1605 (m), 1595 (s), 1247 (s), 1209 (m), 1185 (m), 1036 (m), 822 (s), 747 (m), 664 (s); δ_H ($CDCl_3$, 500 MHz): 1.89 (d, *J* = 6.9 Hz, 3H), 3.84 (s, 3H), 5.37 (q, *J* = 6.9 Hz, 1H), 6.96 (bs, 1H), 6.97 (d, *J* = 9.0 Hz, 2H),

7.10 (bs, 1H), 7.18 (d, *J* = 8.2 Hz, 2H), 7.49 (d, *J* = 9.0 Hz, 2H), 7.52 (d, *J* = 8.2 Hz, 2H), 7.61 (bs, 1H); δ_C ($CDCl_3$, 125 MHz): 22.0 (CH_3), 55.3 (CH), 56.3 (CH_3), 114.3 (CH), 117.9 (CH), 126.4 (CH), 127.1 (CH), 128.1 (CH), 129.4 (CH), 132.8 (C_q), 136.1 (CH), 139.8 (C_q), 140.7 (C_q), 159.4 (C_q); MS (ESI): *m/z* = 279 [M^++H].

5.4.7.2. 1-[1-(3'-Methoxy-biphenyl-4-yl)-ethyl]-1H-imidazole (2)

Synthesised according to Method F using **2a** (0.39 g, 1.7 mmol) and CDI (0.55 g, 3.4 mmol) in acetonitrile; colourless oil; yield: 0.21 g (44 %); R_f = 0.31 (EtOAc / MeOH, 95:5); IR (ATR) $\tilde{\upsilon}$ (cm^{-1}): 1599 (w), 1566 (w), 1481 (m), 1296 (m), 1260 (m), 1220 (s), 1031 (s), 1013 (s), 787 (s); δ_H ($CDCl_3$, 500 MHz): 1.89 (d, *J* = 6.9 Hz, 3H), 3.85 (s, 3H), 5.39 (q, *J* = 6.9 Hz, 1H), 6.90 (ddd, *J* = 1.0, 2.5, 8.2 Hz, 1H), 6.97 (bs, 1H), 7.08 (dd, *J* = 1.6, 2.5 Hz, 1H), 7.09 (bs, 1H), 7.14 (ddd, *J* = 1.0, 1.6, 7.9 Hz, 1H), 7.20 (d, *J* = 8.2 Hz, 2H), 7.35 (t, *J* = 7.9 Hz, 1H), 7.55 (d, *J* = 8.2 Hz, 2H), 7.63 (bs, 1H); δ_C ($CDCl_3$, 125 MHz): 21.0 (CH_3), 55.3 (CH_3), 56.3 (CH), 112.8 (CH), 112.9 (CH), 118.0 (CH), 119.5 (CH), 126.4 (CH), 127.6 (CH), 129.3 (CH), 129.8 (CH), 136.0 (CH), 140.5 (C_q), 141.0 (C_q), 141.8 (C_q), 160.0 (C_q); MS (ESI): *m/z* = 279 [M^++H].

1-[1-(4'-Methoxy-biphenyl-4-yl)-propyl]-1H-imidazole (9)

Synthesised according to Method F using **9a** (2.12 g, 8.8 mmol) and CDI (7.29 g, 45.0 mmol) in NMP; yellow solid; yield: 0.28 g (11 %) R_f = 0.52 (EtOAc / NH_3 (aq, 25%) 97.5:2.5); IR (ATR) $\tilde{\upsilon}$ (cm^{-1}): 2962 (w), 2932 (w), 1605 (m), 1497 (s), 1254 (s), 818 (s); δ_H ($CDCl_3$, 500 MHz): 0.97 (t, *J* = 7.3 Hz, 3H), 2.24 – 2.30 (m, 2H), 3.84 (s, 3H), 5.10 (t, *J* = 7.6 Hz, 1H), 6.96 (d, *J* = 8.5 Hz, 2H), 7.00 (bs, 1H), 7.13 (bs, 1H), 7.25 (d, *J* = 8.5 Hz, 2H), 7.49 (d, *J* = 8.5 Hz, 2H), 7.52 (d, *J* = 8.5 Hz, 2H), 7.89 (bs, 1H); δ_C ($CDCl_3$, 125 MHz): 11.0 (CH_3), 28.4 (CH_2), 55.3 (O-CH_3), 63.4 (CH), 114.3 (CH), 117.9 (CH), 127.0 (CH), 127.1 (CH), 127.9 (C_q), 128.0 (CH), 132.7 (C_q), 136.0 (CH), 137.9 (C_q), 140.9 (C_q), 159.4 (C_{OMe}); MS (ESI): *m/z* = 293 [M^++H].

1-[1-(3'-Methoxy-biphenyl-4-yl)-propyl]-1H-imidazole (10)

Synthesised according to Method F using **10a** (2.30 g, 9.0 mmol) and CDI (7.29 g, 45.0 mmol) in NMP; yellow solid; yield: 0.89 g (30 %); R_f = 0.52 (EtOAc / NH_3 (aq, 25%) 97.5:2.5); IR (ATR) $\tilde{\upsilon}$ (cm^{-1}): 2967 (w), 2935 (w), 1503 (s), 1249 (s), 1218 (s), 1024 (s), 806 (s); δ_H ($CDCl_3$, 500 MHz): 0.95 (t, *J* = 7.3 Hz, 3H), 2.22 – 2.28 (m, 2H), 3.84 (s, 3H), 5.05 (t, *J* = 7.9 Hz, 1H), 6.89 (dd, *J* = 2.2, 8.2 Hz, 1H), 6.98 (bs, 1H), 7.08 (t, *J* = 2.2 Hz, 1H), 7.10 (bs, 1H), 7.13 (d, *J* = 7.9 Hz, 1H), 7.24 (d, *J* = 7.9 Hz, 2H), 7.34 (t, *J* = 7.9 Hz, 1H), 7.54 (d, *J* = 8.2 Hz, 2H), 7.69 (bs, 1H); δ_C ($CDCl_3$, 125 MHz): 11.0 (CH_3), 28.5 (CH_2), 55.2 (CH_3-O), 63.0 (CH), 112.8 (CH), 112.8 (CH), 117.7 (CH), 119.5 (CH), 126.0 (CH), 127.5 (CH), 129.0 (C_q), 129.8 (CH), 136.2 (C_q), 139.2 (C_q), 140.9 (C_q), 141.7 (CH), 159.9 (C_{OMe}); MS (ESI): *m/z* = 293 [M^++H].

1-[1-(3',4'-Dimethoxy-biphenyl-4-yl)-propyl]-1H-imidazole (11)

Synthesised according to Method F using **11a** (2.41 g, 8.90 mmol) and CDI (7.29 g, 45.0 mmol) in NMP; amber oil; yield: 0.69 g (24 %); R_f = 0.47 (EtOAc / NH_3 (aq, 25%) 97.5:2.5); IR (ATR) $\tilde{\upsilon}$ (cm^{-1}): 2967 (w), 2937 (w), 2875 (w), 2838 (w), 1668 (m), 1604 (m), 1583 (m), 1480 (s), 1294 (s), 1101 (s),

816 (s), 778 (s), 743 (s); δ_H ($CDCl_3$, 500 MHz): 0.99 (t, *J* = 7.3 Hz, 3H), 2.28- 2.35 (m, 2H), 3.92 (s, 3H), 3.94 (s, 3H), 5.21 (t, *J* = 7.9 Hz, 1H), 6.94 (d, *J* = 8.2 Hz, 1H), 7.05 (bs, 1H), 7.07 (d, *J* = 2.2 Hz, 1H), 7.11 (dd, *J* = 2.2, 8.2 Hz, 1H), 7.21 (bs, 1H), 7.30 (d, *J* = 8.2 Hz, 2H), 7.55 (d, *J* = 8.2 Hz, 2H), 8.29 (bs, 1H); δ_C ($CDCl_3$, 125 MHz): 11.0 (CH_3), 28.5 (CH_2), 55.9 (O-CH_3), 55.9 (O-CH_3), 62.9 (CH), 110.3 (CH), 111.4 (CH), 117.6 (CH), 119.3 (CH), 126.9 (CH), 127.1 (CH), 129.4 (CH), 133.2 (C_q), 136.3 (CH), 138.7 (C_q), 140.8 (C_q), 148.8 (C_q), 149.1 (C_q); MS (ESI): *m/z* = 323 [M^++H].

1-[1-(4'-Ethoxy-biphenyl-4-yl)-propyl]-1H-imidazole (12)

Synthesised according to Method F using **12a** (1.14 g, 4.45 mmol) and CDI (3.61 g, 22.3 mmol) in NMP; brown solid; yield: 0.60 g (45 %); R_f = 0.66 (EtOAc / MeOH 95:5); IR (ATR) $\tilde{\upsilon}$ (cm^{-1}): 3115 (w), 2976 (w), 2936 (w), 2877 (w), 1606 (m), 1497 (s), 1245 (s), 1043 (s), 827 (s), 815 (s), 784 (s), 740 (s), 665 (s); δ_H ($CDCl_3$, 500 MHz) 0.95 (t, *J* = 7.3 Hz, 3H), 1.42 (t, *J* = 6.9 Hz, 3H), 2.22 - 2.29 (m, 2H), 4.07 (q, *J* = 6.9 Hz, 2H), 5.03 (t, *J* = 7.7 Hz, 1H), 6.95 (d, *J* = 8.8 Hz, 2H), 6.97 (bs, 1H), 7.09 (bs, 1H), 7.22 (d, *J* = 8.2 Hz, 2H), 7.48 (d, *J* = 8.8 Hz, 2H), 7.52 (d, *J* = 8,2 Hz, 2H), 7.63 (bs, 1H); δ_C ($CDCl_3$, 125 MHz): 11.1 (CH_3), 14.8 (CH_3), 28.6 (CH_2), 63.1 (CH), 63.5 (CH_2), 114.8 (CH), 117.7 (CH), 126.9 (CH), 127.0 (CH), 128.0 (CH), 129.3 (C_q), 132.6 (CH), 136.3 (CH), 138.4 (C_q), 140.7 (C_q), 158.7 (C_q); MS (ESI): *m/z* = 307 [M^++H].

Method G: CDI reaction and Deprotection with TBAF (Compounds 3 – 8, 13 - 15)

To a solution of the corresponding alcohol (1.0 eq) in NMP (10 mL / mmol) CDI (5.0 eq) was added at rt. The solution was heated to reflux for 4 to 18 h. After cooling to ambient temperature,the reaction miture was diluted with water (30 mL) and extracted with ethyl acetate (3 x 10 mL). The combined organic phases were washed with brine, dried over $MgSO_4$ and evaporated under reduced pressure. The crude intermediate of the silyl-protected phenol was directly diluted in anhydrous THF, tetrabutylammonium fluoride solution was added (1.0 M in THF, 1.1 eq per TBDMS), and the reaction mixture was stirred for 4 h. The reaction was quenched by addition of methanol, and the solvent was removed under reduced pressure. The crude product was purified by chromatography using silica gel.

4'-(1-Imidazol-1-yl-propyl)-biphenyl-4-ol (3)

Synthesised according to Method G using **3a** (0.85 g, 2.16 mmol); yellow solid; yield: 0.22 g (37 %); R_f = 0.21 (hexane / EtOAc, 5:1); IR (ATR) $\tilde{\upsilon}$ (cm^{-1}): 2963 (w), 2930 (w), 2364 (w),1607 (m), 1588 (m), 1498 (s), 1272 (m), 1070 (s), 808 (s), 748 (m), 658 (m); δ_H (DMSO-d_6, 500 MHz): 0.82 (t, *J* = 7.3 Hz, 3H), 2.21 – 2.25 (m, 2H), 5.23 (t, *J* = 7.3 Hz, 1H), 6.82 (d, *J* = 8.8 Hz, 2H), 6.90 (s, 1H), 7.37 (d, *J* = 8.5 Hz, 2H), 7.46 (d, *J* = 8.8 Hz, 2H), 7.54 (d, *J* = 8.5 Hz, 2H), 7.82 (s, 1H), 9.55 (s, 1H); δ_C (DMSO-d_6, 125 MHz): 10.9 (CH_3), 27.4 (CH_2), 61.5 (CH), 115.6 (CH), 117.7 (CH), 126.0 (CH), 127.1 (CH), 127.6 (CH), 128.4 (C_q), 130.3 (CH), 130.7 (C_q), 139.3 (CH), 139.5 (C_q), 157.1 (C_q); MS (ESI): *m/z* = 279 [M^++H].

4'-(1-Imidazol-1-yl-propyl)-biphenyl-3-ol (4)

Synthesised according to Method G using **4a** (1.32 g, 3.36 mmol); yellow solid; yield: 0.20 g (21 %); R_f = 0.13 (EtOAc / MeOH, 9:1); IR (ATR) $\tilde{\upsilon}$ (cm^{-1}) 2963 (w), 2361 (w), 1583 (m), 1564 (m), 1477 (s), 817 (s), 781 (s), 740 (s); δ_H (DMSO-d_6, 500 MHz): 0.83 (t, *J* = 7.3 Hz, 3H), 2.17 – 2.22 (m, 2H), 5.24 – 5.26 (m, 1H), 6.73 – 6.76 (m, 1H), 6.91 (bs, 1H), 6.99 (t, *J* = 2.2 Hz, 1H), 7.04 (ddd, *J* = 0.9, 2.2, 7.6 Hz, 1H), 7.23 (t, *J* = 7.6 Hz, 1H), 7.33 (t, *J* = 1.3 Hz, 1H), 7.41 (d, *J* = 8.2 Hz, 2H), 7.56 (d, *J* = 8.2 Hz, 2H), 7.83 (t, *J* = 0.9 Hz, 1H), 9.55 (s, 1H); δ_C (DMSO-d_6, 125 MHz): 10.9 (CH_3), 27.4 (CH_2), 61.5 (CH), 113.4 (CH), 114.4 (CH), 117.4 (CH) 117.7 (CH), 126.7 (CH), 127.1 (CH), 128.5 (CH), 129.8 (CH), 136.4 (CH), 140.4 (C_q), 141.0 (C_q), 157.7 (C_{OH}); MS (ESI): *m/z* = 279 [M^++H].

4'-(1-Imidazol-1-yl-propyl)-biphenyl-3,4-diol (5)

Synthesised according to Method G using **5a** (1.00 g, 1.92 mmol); yellow solid; yield: 0.19 g (34 %); R_f = 0.31 (EtOAc / MeOH. 95:5); IR (ATR) $\tilde{\upsilon}$ (cm^{-1}): 3285 (m), 3157 (m), 2963 (m), 2931 (m), 2874 (m), 2828 (m), 1770 (m), 1952 (s), 1945 (s), 1495 (s), 1304 (s), 1219 (s), 1086 (s), 805 (s), 755 (s); δ_H (DMSO-d_6, 500 MHz): 0.81 (t, *J* = 7.3 Hz, 3H), 2.18 – 2.24 (m, 2H), 5.20 – 5.23 (m, 1H), 6.79 (d, *J* = 8.2 Hz, 1H), 6.88 – 6.92 (m, 2H), 7.00 (d, *J* = 2.2 Hz, 1H), 7.31 (t, *J* = 1.3 Hz, 1H), 7.35 (d, *J* = 8.2 Hz, 2H), 7.48 (d, *J* = 8.2 Hz, 2H), 7.82 (bs, 1H), 9.01 (bs, 1H), 9.06 (bs, 1H); MS (ESI): *m/z* = 295 [M^++H].

4'-(1-Imidazol-1-yl-propyl)-biphenyl-3,5-diol (6)

Synthesised according to Method G using **6a** (2.00 g, 3.83 mmol); yellow solid; yield: 0.06 g (5 %); R_f = 0.38 (EtOAc / MeOH, 95 :5); IR (ATR) $\tilde{\upsilon}$ (cm^{-1}): 2975 (w), 1595 (m), 1488 (m), 1351 (m), 1167 (s), 1111 (m), 1091 (m), 1077 (m), 1014 (m), 831 (s), 820 (s), 803 (s), 742 (s); δ_H (DMSO-d_6, 500 MHz) 0.82 (t, *J* = 7.3 Hz, 3H), 2.15 - 2.28 (m, 2H), 5.23 – 5.26 (m, 1H), 6.21 (t, *J* = 2.1 Hz, 1H), 6.44 (d, *J* = 2.1 Hz, 2H), 6.91 (s, 1H), 7.31 (s, 1H), 7.38 (d, *J* = 8.2 Hz, 2H), 7.49 (d, *J* = 8.2 Hz, 2H), 7.82 (s, 1H), 9.36 (s, 2H); δ_C (DMSO-d_6, 125 MHz): 10.9 (CH_3), 27.5 (CH_2), 61.5 (CH), 101.7 (CH), 104.7 (CH), 117.7 (CH), 126.6 (CH), 127.0 (CH), 128.5 (CH), 132.5 (C_q), 136.4 (CH), 139.9 (C_q), 141.5 (C_q), 158.7 (C_{OH}); MS (ESI): *m/z* = 295 [M^++H].

3'-Fluoro-4'-(1-imidazol-1-yl-propyl)-biphenyl-4-ol (7)

Synthesised according to Method G using **7a** (crude product); yellow solid; yield: 0.05 g; R_f = 0.4 (EtOAc / MeOH, 95:5); IR (ATR) $\tilde{\upsilon}$ (cm^{-1}): 2974 (w), 2360 (m), 2341 (m), 1607 (m), 1494 (s), 1283 (m), 1223 (m), 1077 (m), 840 (m), 827 (s), 765 (m); δ_H (DMSO-d_6, 500 MHz): 0.84 (t, *J* = 7.3 Hz, 3H), 2.14 – 2.32 (m, 2H), 5.47 – 5.50 (m, 1H), 6.83 (d, *J* = 8.5 Hz, 2H), 6.90 (bs, 1H), 7.30 (t, *J* = 1.3 Hz, 1H), 7.41 – 7.44 (m, 3H), 7.51 (d, *J* = 8.5 Hz, 2H), 7.81 (bs, 1H), 9.67 (s, 1H); MS (ESI): *m/z* = 297 [M^++H].

3'-Fluoro-4'-(1-imidazol-1-yl-propyl)-biphenyl-3,4-diol (8)

Synthesised according to Method G using **8a** (crude product); yellow solid; yield: 0.05 g; R_f = 0.45 (EtOAc/MeOH, 95:5); IR (ATR) $\tilde{\upsilon}$ (cm^{-1}): 1500 (s), 1413 (m)1279 (m), 1264 (s), 1109 (s), 1089 (s), 859 (s), 812 (s), 785 (m), 742 (m); δ_H ($CDCl_3$, 500 MHz): 1.00 (t, *J* = 7.3 Hz, 3H), 2.26 – 2.32 (m,

2H), 5.35 (t, *J* = 7.9 Hz, 1H), 6.93 – 6.97 (m, 2H), 7.01 (t, *J* = 1.3 Hz, 1H), 7.03 (d, *J* = 1.9 Hz, 1H), 7.10 (t, *J* = 1.3 Hz, 1H), 7.14 (dd, *J* = 1.9, 12.0 Hz, 1H), 7.17 (d, *J* = 7.9 Hz, 1H), 7.24 (dd, *J* = 1.9, 8.2 Hz, 1H), 7.78 (s, 1H); MS (ESI): *m/z* = 313 [M^++H].

4'-(1-Imidazol-1-yl-propyl)-3,5-dimethyl-biphenyl-4-ol (13)

Synthesised according to Method G using **13a** (0.30 g, 0.71 mmol) and TBAF solution (1.0 M in THF, 0.78 mL, 0.78 mmol); white solid; yield: 0.19 g (85 %); R_f = 0.22 (hexane / EtOAc, 5:1); IR (ATR) $\tilde{\upsilon}$ (cm^{-1}): 2927 (w), 1705 (w), 1484 (s), 1188 (m), 1076 (w), 923 (m), 817 (w), 731 (s), 662 (s), 543 (w); δ_H ($CDCl_3$, 500 MHz): 0.95 (t, *J* = 7.3 Hz, 3H, CH_3), 2.23 – 2.26 (m, 2H), 2.31 (s, 6H), 5.04 (t, *J* = 7.6 Hz, 1H), 6.97 (s, 1H), 7.09 (s, 1H), 7.18 (s, 2H), 7.20 (d, *J* = 8.2 Hz, 2H), 7.49 (d, *J* = 8.2 Hz, 2H), 7.63 (s, 1H); δ_C ($CDCl_3$, 125 MHz): 11.1 (CH_3), 16.2 (CH_2), 28.6 (CH_3), 30.9 (CH_3), 63.1 (CH), 117.7 (CH), 126.8 (C_q), 127.1 (CH), 127.2 (CH), 129.3 (CH), 136.3 (C_q), 138.2 (C_q), 141.1 (C_q); MS (ESI): *m/z* = 307 [M^++H].

4'-(1-Imidazol-1-yl-propyl)-3-methyl-biphenyl-4-ol (14)

Synthesised according to Method G using **14a** (0.24 g, 0.59 mmol) and TBAF solution (1.0 M in THF, 0.89 mL, 0.89 mmol); white solid; yield: 0.14 g (83 %); R_f = 0.22 (hexane / EtOAc, 5:1); IR (ATR) $\tilde{\upsilon}$ (cm^{-1}): 2933 (w), 2361 (w), 1602 (m), 1499 (s), 1398 (m), 1278 (s), 1129 (w), 1087 (w), 1075 (m), 927 (w), 815 (s), 739 (m), 659 (m); δ_H ($CDCl_3$, 500 MHz): 0.97 (t, *J* = 7.3 Hz, 3H), 2.23 – 2.29 (m, 2H), 2.32 (s, 3H), 5.04 (t, *J* = 7.6 Hz, 1H), 6.81 (d, *J* = 8.3 Hz, 1H), 7.01 (s, 1H), 7.13 (s, 1H), 7.19-7.23 (m, 3H), 7.33 (d, *J* = 1.9 Hz, 1H), 7.50 (d, *J* = 8.3 Hz, 2H), 7.67 (s, 1H); δ_C ($CDCl_3$, 125 MHz): 11.1 (CH_3), 16.2 (CH_2), 28.5 (CH_3), 63.3 (CH), 115.2 (CH), 117.9 (CH), 124.8 (CH), 125.4 (CH), 126.8, 127.0 (CH), 128.9 (CH), 129.6 (CH), 131.1 (CH), 136.2 (C_q), 137.9 (C_q), 141.2 (C_q), 154.9 (C_{OH}); MS (ESI): *m/z* = 293 [M^++H].

3-Chloro-4'-(1-imidazol-1-yl-propyl)-biphenyl-4-ol (15)

Synthesised according to Method G using **15a** (0.27 g, 0.63 mmol) and TBAF solution (1.0 M in THF, 0.91 mL, 0.91 mmol); white solid; yield: 0.18 g (86 %); R_f = 0.25 (hexane / EtOAc, 5:1); IR (ATR) $\tilde{\upsilon}$ (cm^{-1}): 2360(m), 1497 (s), 1293 (s), 1054 (m), 810 (s), 735 (m), 659 (m); δ_H ($CDCl_3$, 500 MHz): 0.96 (t, *J* = 7.3 Hz, 3H), 2.23 - 2.29 (m, 2H), 5.04 (t, *J* = 7.3 Hz, 1H), 6.99 (d, *J* = 8.3 Hz, 1H), 7.04 (s, 1H), 7.13 (s, 1H), 7.19-7.23 (m, 3H), 7.47 (d, *J* = 1.9 Hz, 1H), 7.52 (d, *J* = 8.3 Hz, 2H), 7.80 (s, 1H); δ_C ($CDCl_3$, 125 MHz): 11.0 (CH_3), 28.4 (CH_2), 63.5 (CH), 116.9 (CH), 118.1 (CH), 126.6 (CH), 127.0 (CH), 127.1(CH), 127.9 (CH), 135.8 (C_q), 139.9 (C_q); MS (ESI): *m/z* = 313 [M^++H].

Docking studies

Ligands

All molecular modelling studies were performed on Intel(R) P4 CPU 3.00GHz running Linux Suse 10.1. The structures of the inhibitors were built with SYBYL 7.3.2 (Sybyl, Tripos Inc., St. Louis, Missouri, USA) and energy-minimized in MMFF94s force-field [43] as implemented in Sybyl. The resulting geometries for our compounds were then subjected to *ab initio* calculation employing the

B3LYP functional [44, 45] in combination with a 6-31G* basis set using the package Gaussian03 (Gaussian, Inc., Pittsburgh, PA, 2003).

Docking

Molecular docking calculations were performed for various inhibitors of Table 1. Since the GOLD docking program allows flexible docking of the compounds, no conformational search was employed to the ligand structures. GOLD gave the best poses by a genetic algorithm (GA) search strategy, and then various molecular features were encoded as a chromosome.

Ligands were docked in 50 independent genetic algorithm (GA) runs using GOLD. Heme iron was chosen as active-site origin, while the radius was set equal to 19 Å. The automatic active-site detection was switched on. A distance constraint of a minimum of 1.9 and a maximum of 2.5 Å between the sp^2-hybridised nitrogen of the imidazole and the iron was set. Further, some of the GOLDSCORE parameters were modified to improve the weight of hydrophobic interaction and of the coordination between iron and nitrogen. The genetic algorithm default parameters were set as suggested by the GOLD authors [41]. On the other hand, the annealing parameters of fitness function were set at 3.5 Å for hydrogen bonding and 6.5 Å for Van der Waals interactions.

All 50 poses for each compound were clustered with ACIAP [46, 47] and the representative structure of each significant cluster was selected. The quality of the docked representative poses was evaluated based on visual inspection of the putative binding modes of the ligands, as outcome of docking simulations and cluster analysis.

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Appendix. Supplementary information. Supplementary data regarding CYP3A4 and CYP11B1 (one table) can be found in the online version, at doi: 10.1016/j.ejmech.2009.01.002.

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3.2 CYP17 Inhibitors. Annulations of Additional Rings in Methylene Imidazole Substituted Biphenyls: Synthesis, Biological Evaluation and Molecular Modelling

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Paper II

Abstract:

Twenty-one novel compounds originating from two classes of annulated biphenyls were synthesised as mimetics of the steroidal A- and C-rings and examined for their potency as inhibitors of human CYP17. Selected compounds were tested for inhibition of the hepatic CYP enzyme 3A4. Potent CYP17 inhibitors were found for each class, compound **9** (17 and 71% at 0.2 and 2 µM, respectively) and **21** (591 nM). Compound **21** showed only weak inhibition of CYP3A4 (32 and 64% at 2 and 10 µM, respectively). Both compounds, however, exhibited moderate to strong inhibition of the glucocorticoid-forming enzyme CYP11B1. The most interesting compounds were docked into our protein model. They bound into one of the modes which we have previously published. New interaction regions were identified.

Introduction:

Prostate cancer is a major cause of death in elderly men worldwide.[1] It is widely demonstrated that high androgen levels (testosterone and dihydrotestosterone) stimulate tumor growth in prostate cancer.[2] Thus, androgen receptor antagonists[3] and gonadotropin-releasing hormone analogues[4] are used as a standard therapy. The major drawback of these therapies is the fact that they do not reduce androgen concentrations or only affect testicular androgen production, allowing androgens still to be produced in the adrenals.

Therefore, a new promising target is 17α-hydroxylase-17, 20-lyase (CYP17), the key enzyme for the biosynthesis of androgens. It is catalyzing the conversion of pregnenolone or progesterone to DHEA or androstenedione, respectively. Even more, this target has already clinically proven success with the antimicotic ketoconazole which is also a weak inhibitor of CYP17.[5] In previous works, we could demonstrate in-vitro and in-vivo activity for steroidal[6] and non-steroidal[7-10, 11] compounds. Some of these compounds had been designed as mimetics of the steroidal AC-rings (Chart 1).[10, 11] Since they had shown a high activity and a good selectivity, we chose them for further optimizations.

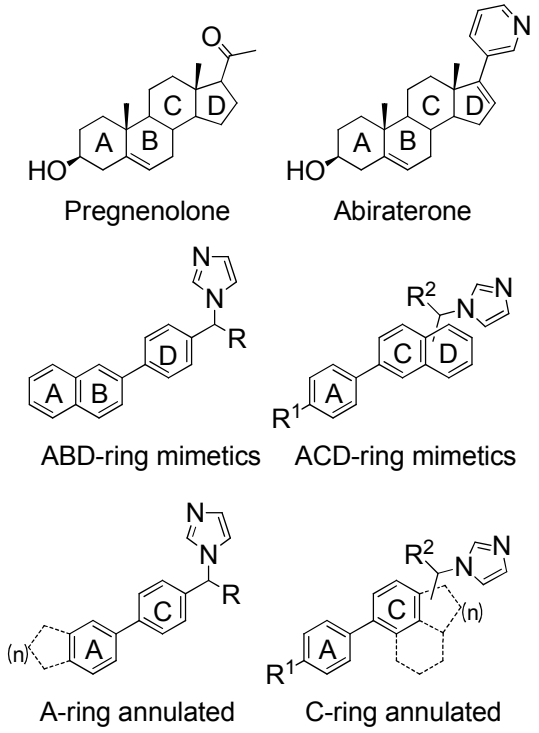
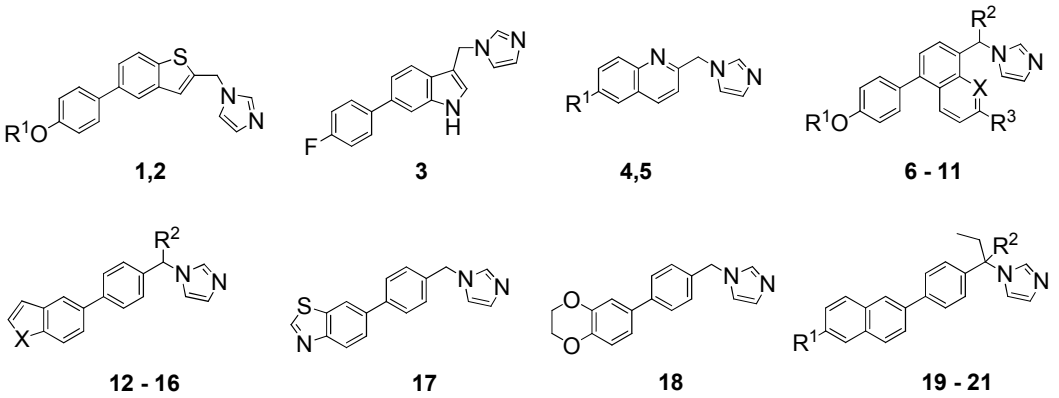


Chart 1. Substrate, abiraterone, described ABD- and ACD-ring mimetic CYP17 inhibitors and A- and C-ring annulated compounds of the present study.

Very recently,[12] we found new highly potent and selective compounds, which showed better pharmacokinetic and pharmacodynamic profiles than abiraterone, a CYP17 inhibitor currently

undergoing clinical phase II,[13] by replacing the A-ring-mimicking benzene nucleus with different heterocycles.

In order to further explore the spatial limitations surrounding the A- and C-ring binding regions, in this work, we expand the corresponding biphenyl rings by annulations of different aromatic and non-aromatic rings. In this way, two different compound classes were synthesised (Chart 2), by either annealing the C-ring (compounds **1**-**11**) or by otherwise annealing the A-ring (compounds **12**-**21**).



Compound	R^1	R^2	R^3	X
1	Me			
2	H			
4	p-F-(C_6H_4)			
5	3-thiophenyl			
6	Me	H	H	CH
7	H	H	H	CH
8	Me	Et	H	N
9	H	Et	H	N
10	Me	Et	Et	N
11	H	Et	Et	N
12		Et		S
13		Et		NH
14		H		O
15		H		S
16		H		NH
19	OMe	H		
20	H	H		
21	OH	Et		

Chart 2. List of synthesizsed compounds **1**-**21**.

Like in previous works,[10-12] 1-imidazole linked with a methylene spacer was introduced as nitrogen-bearing heterocycle, since the complexation of the heme iron by an aromatic nitrogen is an important prerequisite for inhibition of cytochrome P450 enzymes.[14] We have also shown[10-12] that the introduction of a fluorine, hydroxy, and methoxy group in the A-ring strongly contributed to a better inhibition of our target enzyme.

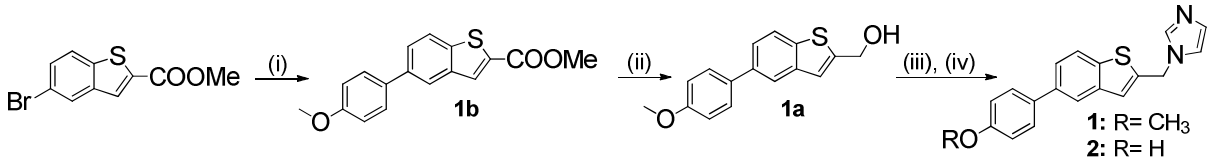
In the following, the synthesis, CYP17 inhibitory activities, and molecular modelling studies are presented and these data are compared to the ones recently obtained with ABD- and ACD-ring

mimetics (Chart 1).[9] Besides, for reasons of selectivity, inhibition of the most crucial hepatic CYP enzyme CYP3A4 was monitored, and for selected compounds inhibition of the glucocorticoid-forming enzyme CYP11B1 was also determined. The most promising compounds were docked into our protein model, and the key interactions with the enzyme were elucidated.

Chemistry:

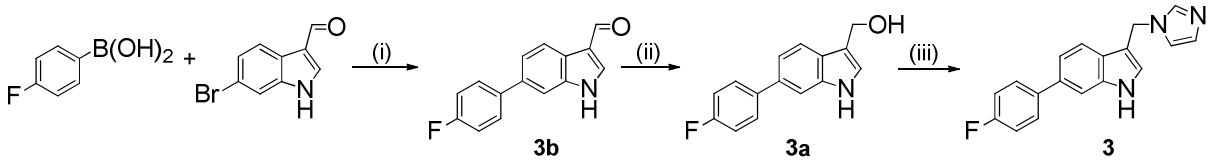
The syntheses of compounds **1**-**21** are shown in Schemes 1-7. In pursuing our aim to explore the binding regions surrounding the A- and C-rings, different aromatic and non-aromatic moieties were annulated to the A (Schemes 1-5) or C (Schemes 6, 7) ring. The coupling of the biphenylic moiety was achieved by means of Suzuki coupling[15] (Method C) except for the synthesis of compounds **1** and **2** (Scheme 1) where a Negishi coupling had to be applied. When the necessary bromides for the couplings were not commercially available, they were prepared by bromination using NBS (N-bromosuccinimide) (Scheme 3). The imidazoles were introduced by performing a S_{Nt} reaction with 1,1'-carbonyldiimidazole (CDI) and the corresponding alcohol[16] (Method A) or via S_{N2} reaction of an alkyl bromide with imidazole (Scheme 3).

The alcohols were obtained from either the carboxylic derivatives (Schemes 1, 4) or from the aldehydes (Methods D, E). In most cases, the methoxy-substituted compounds were submitted to an ether cleavage (Method B). For the preparation of compound **21**, the hydroxyl group on the naphthalene had to be protected before the Suzuki coupling due to otherwise very low yields.[15]



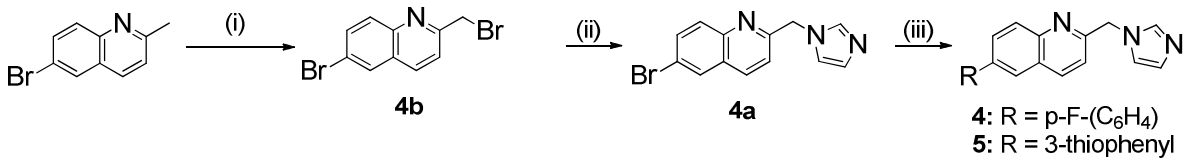
Scheme 1. Synthesis route of compounds 1 and 2

Reagent and conditions: (i) a: 4-bromo-anisole, *tert*-BuLi, THF, -78°C, 30 min; b: $ZnCl_2$, 0°C, 30 min; c: $PdCl_2(PPh_3)_2$, 16 h; **(ii)** $LiBH_4$, toluene, THF, Et_2O, 110°C, 16 h; **(iii) Method A1:** CDI, imidazole, NMP, 180°C, 16 h; **(iv) Method B:** BBr_3, DCM, -78°C to 0°C, 16 h.



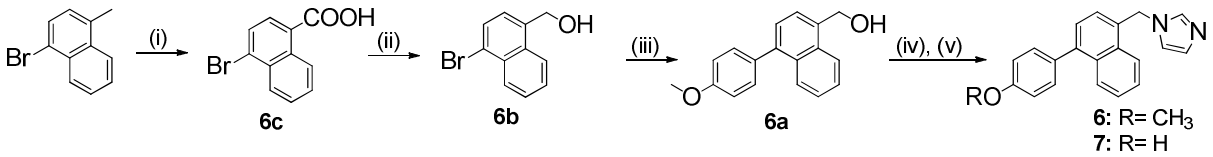
Scheme 2. Synthesis route of compound 3

Reagents and conditions: (i) Method C1: Na_2CO_3, $Pd(PPh_3)_4$, toluene/EtOH/H_2O, reflux, 5 h; **(ii) Method D:** $NaBH_4$, MeOH, rt, 2 h; **(iii) Method A2:** CDI, acetonitrile, reflux, 18 h.



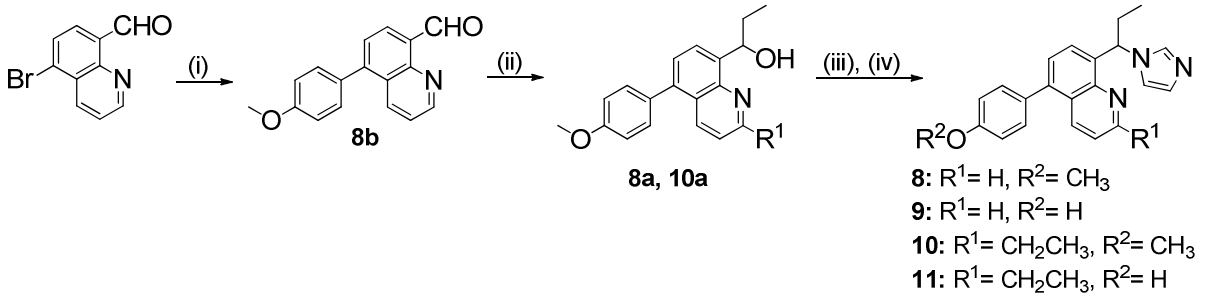
Scheme 3. Synthesis route of compounds 4 and 5

Reagents and conditions: (i) NBS, CCl_4, 75°C, 16 h; **(ii)** Imidazole, K_2CO_3, 18-crown-6, acetonitrile, 100°C, 16 h; **(iii) Method C1:** 4-fluorophenylboronic acid (**5:** 3-thiophenylboronic acid), Na_2CO_3, $Pd(PPh_3)_4$, toluene/MeOH/H_2O, 70°C, 5 h.



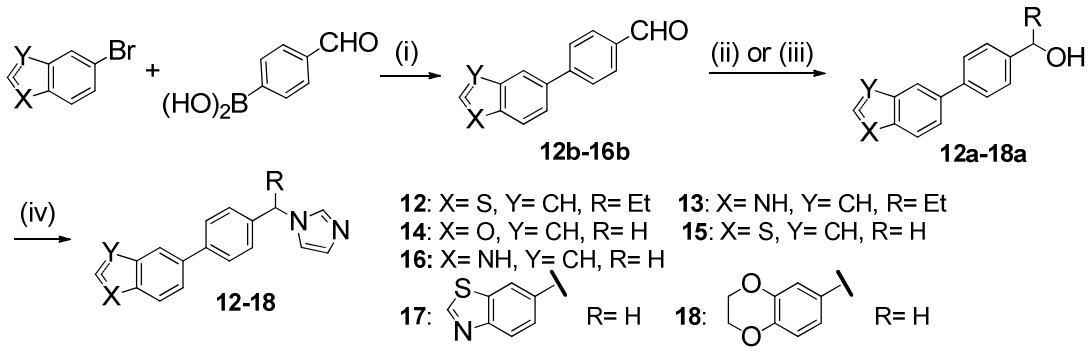
Scheme 4. Synthesis route of compounds 6 and 7

Reagents and conditions: (i) $KMnO_4$, pyridine, H_2O, 50°C, 4 d; **(ii)** $LiAlH_4$, Et_2O, 35°C, 16 h; **(iii) Method C2:** 4-methoxyphenylboronic acid, Cs_2CO_3, $Pd(OAc)_2$, TBAB, toluene/EtOH/H_2O, 110°C, 16 h; **(iv) Method A1:** CDI, imidazole, NMP, 180°C, 2 d; **(v) Method B:** BBr_3, DCM, -78°C to 0°C, 16 h.



Scheme 5. Synthesis route of compounds 8-11

Reagents and conditions: (i) Method C2: 4-methoxyphenylboronic acid, Cs_2CO_3, $Pd(OAc)_2$, TBAB, toluene/EtOH/H_2O, 110°C, 16 h; **(ii) Method E:** EtMgBr, THF, 0°C to rt, 16 h; **(iii) Method A1:** CDI, imidazole, NMP, 180°C, 1-2.5 d; **(iv) Method B:** BBr_3, DCM, -78°C to 0°C, 16 h.



Scheme 6. Synthesis route of compounds 12-18

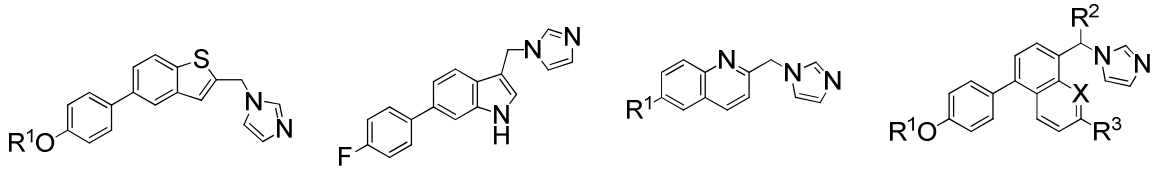
Reagents and conditions: (i) Method C1: $Pd(PPh_3)_4$, TBAB, Na_2CO_3, toluene/EtOH/H_2O, reflux, 16 h; **(ii) Method E: 12a-13a:** EtMgBr, THF, 0°C to rt, 16 h; **(iii) Method D: 14a-18a:** $NaBH_4$, MeOH, reflux, 2 h; **(iv) Method A1:** CDI, NMP, reflux, 3 h.

Results:

Biological Results. Inhibition of CYP17 was evaluated using human enzyme expressed in *E. coli*.[17] The percent inhibition values of the compounds were determined with the 50,000 g sediment of the *E. coli* homogenate, progesterone (25 µM) as substrate and the inhibitors at concentrations of 0.2 and 2.0 µM. Separation of substrate and product was accomplished by HPLC using UV detection.[7]

In contrast to the reference compound ketoconazole, the C-ring-annulated compounds (**1**-**11**, Table 1) mostly showed moderate to no inhibition with exception of the quinoline compound **9**, which showed 71% inhibition at 2 µM. The prolongation of the C-ring in compounds **1**-**5** led to non-active compounds. The quinolines **8**-**11** bearing an ethyl moiety at the methylene linker showed an overall better activity than the naphthalenes **6** and **7**.

Table 1. Inhibition of CYP17 by C-ring annulated compounds **1**-**11**



1, **2** **3** **4**, **5** **6**-**11**

Comp.	Structures				CYP17	
	R^1	R^2	R^3	X	% Inhibition [a]	
					0.2 µM	2 µM
1	Me				0	18
2	H				6	13
3					0	12
4	p-F-(C_6H_4)				1	12
5	3-thiophenyl				0	11
6	Me	H	H	CH	0	5
7	H	H	H	CH	1	12
8	Me	Et	H	N	3	32
9	H	Et	H	N	17	71 [d]
10	Me	Et	Et	N	6	57
11	H	Et	Et	N	5	57
KTZ [b]					19 [c]	

[a] Data shown were obtained by performing the tests in duplicate. The deviations were within < ±5 %. Concentration of progesterone (substrate) was 25 µM.
[b] **KTZ**: ketoconazole.
[c] % inhibition at 1.0 µM.
[d] IC_{50}= 817 nM

The A-ring-annulated compounds (**12**-**21**, Table 2) showed moderate to good activities. However, they did not exceed the activity of compound **9**. The most active compounds in this class showing percent-inhibition values of more than 70% at 2 µM are compound **13** bearing an indole (H-bond donor) and compounds **19** and **21** bearing a methoxy group (H-bond acceptor) or a hydroxyl group (H-bond acceptor and donor), respectively, at the 6-position of a naphthalene. Absence of these

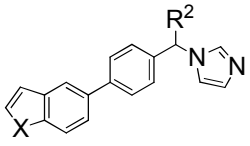
substituents in the latter compounds diminishes the inhibitory activity (compound **20**). The introduction of an ethyl moiety at the methylene linker led to an increase in activity for compounds **15** to **12** and **16** to **13**. All other aromatic heterocycles resulted in only moderate inhibitors.

When comparing the activities of the compounds of this study to the ones of the parent compounds,[11] it must be mentioned that the structural modifications did not increase activities.

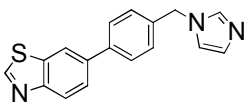
Regarding selectivity against other CYP enzymes, a broader spectrum of our compounds was tested for inhibition of the hepatic enzyme CYP3A4. This enzyme is responsible for the metabolism of lipophilic substances and, therefore, responsible for about 50% of current prescription drugs.[18] While compounds **12**-**19** showed a strong inhibition of this enzyme (>85% inhibition at 2 µM), compound **21** exhibited low inhibitory activity towards CYP3A4 (32% at 2 µM and 64% at 10 µM).

Thus, compound **21** together with the most promising compound **9** of the C-ring-annulated class of compounds, were further tested for inhibition of the steroidogenic enzyme CYP11B1. Its importance relies on the fact that it catalyzes the last step in glucocorticoid formation, namely the transformation of 11-deoxycortisol into cortisol. For the assay,[19] V79MZh11B1 cells expressing human CYP11B1 were used. Both compounds showed strong inhibition of the enzyme at the tested concentrations (**9**: 86 and 94% at 0.2 and 2 µM; **21**: 81 and 95% at 0.2 and 2 µM).

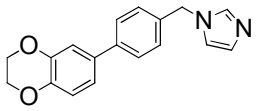
Table 2. Inhibition of CYP17 by A-ring annulated compounds **12**-**21**



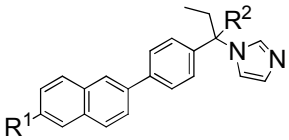
12-**16**



17



18



19-**21**

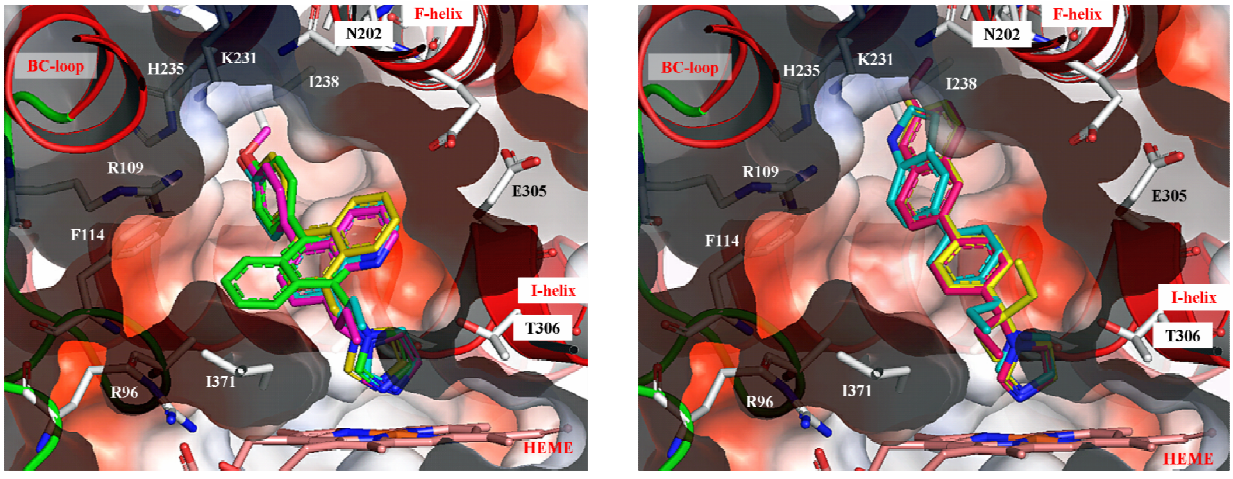
Comp.	Structures			CYP17	
	R^1	R^2	X	% Inhibition [a]	
				0.2 µM	2 µM
12		Et	S	7	40
13		Et	NH	21	75 [d]
14		H	O	5	27
15		H	S	0	21
16		H	NH	2	39
17				5	39
18				0	17
19	OMe	H		19	74 [e]
20	H	H		7	43
21	OH	Et		16	74 [f]
KTZ [b]				19 [c]	

[a] Data shown were obtained by performing the tests in duplicate. The deviations were within < ±5 %. Concentration of progesterone (substrate) was 25 µM.
[b] **KTZ**: ketoconazole.
[c] % inhibition at 1.0 µM.
[d] IC_{50}= 667 nM. [e] IC_{50}= 703 nM. [f] IC_{50}= 591 nM.

Molecular Modelling. Using selected compounds, we explored the binding modes of the A- and C-ring-annulated biphenyls. Several active and less active compounds (C-ring: **6**-**11** (R, S); A-ring: **13** (R, S), **16**, **17**, **19** (R, S) and **21**) were docked by means of the GOLD v 3.0.1 software[20] in the active site of our homology model of CYP17.[12]

Aware of the limitations of docking,[21] the resulting poses of each compound were clustered with AClAP (autonomous hierarchical agglomerative cluster analysis)[22] and the representative poses of each cluster were subjected to a critical visual inspection. H-bonds, π-π, and hydrophobic interactions, as well as steric clashes were measured and evaluated. The necessity of iron-nitrogen complexation[14] for inhibitory activity was also considered. Furthermore, the GOLD v 3.0.1 software, used with a slightly modified GOLDSCORE, was tested on different crystallised CYP enzymes. This program could reproduce quite well the correct orientation of co-crystallised ligands (data not shown). Moreover, GOLD v 3.0.1 produced reliable poses for abiraterone in our CYP17 model,[12] oriented like described for pregnenolone.[23] With these findings, the obtained results can be considered very probable.

All compounds principally showed poses in BM1 – one of two binding modes we have identified for biphenyl type inhibitors[12] – with the modified biaryl-skeleton oriented almost parallel to the I-helix (Fig. 1). Furthermore, the observed increase in activity caused by the ethyl group at the methylene spacer can be explained by the anchoring function of this substituent, namely by hydrophobic interactions with the tiny hydrophobicpocket next to the heme, as already described.[12]



(A) **(B)**

Figure 1. A cross-section of the solvent accessible surface of CYP17 is shown, revealing the active-site cavity with docked: **(A)** C-ring annulated **6** (green)**, 7** (yellow), **8** (magenta) and **9** (cyan) and **(B)** A-ring annulated compounds **13** (cyan), **19** (magenta) and **21** (yellow). Further, heme, interacting residues and ribbon rendered tertiary structure of the active site are shown. Figures were generated with Pymol (http://www.pymol.org).

Regarding the docked C-ring annulated compounds, both enantiomers of compounds **6**-**11** showed mainly one binding mode, except for compounds **6** and **7**, which switch the annealed ring towards or opposite the I-helix (Fig. 1A). The annealed ring is directed towards the kink of the I-helix,[12] stabilised in its orientation by π-π and hydrophobic interactions with the residues Phe114, Gly301-Ala302,

Glu305, Thr306, Ile371, and Val482 (Fig. 1A). Nevertheless, for all three compound couples sterical hindrance, changing in its extend from pair to pair (**8**-**9** < **6**-**7** < **10**-**11**) between the annulated C-ring and the surrounding amino acids, was observed. The extension of the annealed ring system, like the introduction of a space-demanding group, e. g. ethyl was crucial, as seen by the reduced activity of compounds **10** and **11** with respect to **8** and **9**.

For the inhibitory activity of compounds **8**-**11**, the presence of the aromatic nitrogen in the annealed C-ring was striking; it delocalizes the negative charge of the ring system and is capable of H-bond formation with the catalytically important Thr306 and the 1-N of the imidazole.

Compounds **6**-**11** (R, S) showed the ability of forming an H-bond net between the R^1 substituent of the A-ring (Table 1) and the amino acids Arg109 and His235, as it was already observed for their parent compounds.[9] OH showed the highest activity values, leading to the conclusion that an H-bond donor group in this position was necessary.

As for the A-ring annulated compounds, **13** (R, S), **16** (R, S), **17**, **19** (R, S) and **21** were docked into our homology model (Fig 1B). Even these elongated compounds are basically oriented in BM1. However, the extension of the A-ring caused a shift in the interaction area. The substituent R^1 showed H-bond formations with Asn202, Lys231, His235, and, eventually, Arg109, but even hydrophobic interactions between the OMe group in $R^1$and Ile198 and Ile238. Almost the same hydrophobic interactions, as reported previously,[12] between the aromatic core structure and the prevalently hydrophobic surroundings of the active site could be observed. Additionally, the extended A-ring can undergo π-π and hydrophobic interactions with Phe114, Ile205, His235, Gly297, and Thr294. Comparing compounds **13** and **16**-**17**, the presence of a H-bond donor hetero-atom in the annealed A-ring appeared necessary for H-bond formation with His235 and Asn202, with the intent to mimic the para-OH of some of the most active parent compounds.[9]

Discussion and Conclusion:

Similar to our ABD-mimetics,[9] the annulation at the A-ring led mostly to low to moderately active compounds with exception of **9** which showed good inhibition of CYP17. On the other hand, the annealing of an additional ring at the C-ring resulted in moderately to highly active compounds, similar to our findings in the class of ACD-ring mimetic inhibitors.[9]

The best compounds in terms of activity in each class are **9** for the A-ring-expanded and **21** for the C-ring-annulated compounds. Compound **21** is also selective against CYP3A4. Based on both biological results and molecular modelling studies (Fig. 1), we conclude that space occupancy in both the A-ring and the C-ring area is appropriate for the design of new lead structures. The presence of specific heteroatoms, especially N in the annealed rings, is recommended, since this structure modification is capable of H-bond formation and of modifying the electrostatic properties of the annealed ring system. The importance for CYP17 activity of an ethyl substituent at the methylene linker was reiterated as well.

One of our goals in this work was the discovery of a new, more complex lead structure, with the aim of increasing potency and selectivity towards other CYP enzymes. This was achieved with compounds **9** and **21**. For further increasing the activity of compound **9**, we suggest an lead structure optimised by substitution of the annealed ring with a 5-membered aromatic ring bearing a heteroatom, like imidazole, or the exchange of the whole bicyclic structure at the C-ring with a 7-membered ring. These modified compounds are likely to better fit in the active site, due to reduced steric hindrance and hydrophobic repulsion.

Experimental Section

Chemistry.

Melting points were determined on a Mettler FP1 melting point apparatus and are uncorrected. IR spectra were recorded neat on a Bruker Vector 33FT-infrared spectrometer. ^{1}H-NMR spectra were measured on a Bruker DRX-500 (500 MHz). Chemical shifts are given in parts per million (ppm), and TMS was used as an internal standard for spectra obtained in $CDCl_3$. All coupling constants (*J*) are given in Hz. ESI (electrospray ionization) mass spectra were determined on a TSQ quantum (Thermo Electron Corporation) instrument. Elemental analyses were performed at the Department of Instrumental Analysis and Bioanalysis, Saarland University. Column chromatography was performed using silica-gel 60 (50-200 µm), and reaction progress was determined by TLC analysis on Alugram® SIL G/UV_{254} (Macherey-Nagel). Boronic acids and bromoaryls used as starting materials were commercially obtained (CombiBlocks, Chempur, Aldrich, Acros).

Methyl 5-(4-methoxyphenyl)benzo*[b]*thiophene-2-carboxylate, 1b.

To a solution of 1-bromo-4-methoxybenzene (1.32 mL, 10.52 mmol) in dry THF (20 mL) cooled at -78 °C *t*-BuLi (1.5 M, 14.48 mL, 21.72 mmol) was added slowly. After 30 min $ZnCl_2$ (0.5 M, 24.53 mL, 12.26 mmol) was added carefully and after 10 min it was let to warm up to rt. After additional 20 min methyl 5-bromobenzo*[b]*thiophene-2-carboxylate (1.90 g, 7.00 mmol) and bis-(triphenylphosphine)-palladium (II)-dichloride (0.49 g, 0.70 mmol) were prepared in dry THF (30 mL) under protecting atmosphere and the reagent was added at 0 °C and the reaction mixture left stirring overnight. yield: 1.45 g (69 %); R_f = 0.35 (PE / EtOAc, 2:1); δ_H ($CDCl_3$, 500 MHz) 3.87 (s, 3H), 3.96 (s, 3H), 7.01 (d, *J* = 8.8 Hz, 2H), 7.58 (d, *J* = 8.8 Hz, 2H), 7.67 (dd, *J* = 1.8 Hz, *J* = 8.5 Hz, 1H), 7.89 (d, *J* = 8.5 Hz, 1H), 8.01 (s, 1H), 8.09 (s, 1H).

(5-(4-Methoxyphenyl)benzo*[b]*thiophen-2-yl)methanol, 1a.

To a solution of **1b** (0.55 g, 1.84 mmol) in THF (30 mL), toluene (15 mL) and diethyl ether (15 mL) $LiBH_4$ (0.05 g, 2.21 mmol) was added and heated to reflux for 2 h. yield: 0.47 g (95 %); R_f = 0.53 (PE / EtOAc, 2:1); δ_H ($CDCl_3$, 500 MHz) 3.87 (s, 3H), 4.95-5.01 (m, 2H), 6.99-7.01 (m, 3H), 7.53 (dd, *J* = 1.8 Hz, *J* = 8.4 Hz, 1H), 7.56-7.59 (m, 2H), 7.85 (d, *J* = 8.4 Hz, 1H), 7.88 (s, 1H).

Method A: CDI reaction.

To a solution of the corresponding alcohol (1 eq) in NMP or acetonitrile (10 mL / mmol) was added CDI (5 eq). Then the solution was heated to reflux for 4 to 18 h. After cooling to ambient temperature, it was diluted with water (30 mL) and extracted with ethyl acetate (3 x 10 mL). The combined organic phases were washed with brine, dried over $MgSO_4$ and evaporated under reduced pressure. Then the desired product was purified by chromatography on silica gel.

1-((5-(4-Methoxyphenyl)benzo*[b]*thiophen-2-yl)methyl)-*1H*-imidazole, 1.

Synthesised according to Method A1 using **1a** (0.25 g, 0.93 mmol) and CDI (1.20 g, 7.40 mmol); yield: 0.20 g (68 %); white solid: mp 169 °C; R_f = 0.44 (EtOAc / MeOH, 95:5); IR (ATR) υ (cm^{-1}) 3106 (w), 1607 (w), 1516 (m), 1453 (w), 1436 (w), 1277 (m), 1254 (m), 1231 (m), 1194 (w), 1072 (w), 1031 (m), 1015 (w), 908 (w), 806 (s), 744 (s), 667 (m); δ_H ($CDCl_3$, 500 MHz) 3.86 (s, 3H), 5.35 (s, 2H), 6.99-7.01 (m, 3H), 7.12 (s, 1H), 7.17 (s, 1H), 7.52-7.56 (m, 3H), 7.62 (s, 1H), 7.80 (d, *J* = 8.4 Hz, 1H), 7.86 (s, 1H); δ_C ($CDCl_3$, 125 MHz) 46.3, 55.3, 114.3, 119.1, 121.5, 122.6, 123.3, 124.2, 128.3, 130.0, 133.4, 137.2, 137.9, 138.3, 139.8, 139.8, 159.1.

3-((*1H*-Imidazol-1-yl)methyl)-6-(4-fluorophenyl)-*1H*-indole, 3.

Synthesised according to Method A2 using **3a** (0.25 g, 1.04 mmol) and CDI (0.34 g, 2.08 mmol); yield: 0.15 g (52 %); brown solid: mp 171-173 °C; R_f = 0.24 (EtOAc / MeOH, 95:5); δ_H ($CDCl_3$, 500 MHz) 5.29 (s, 2H), 6.91 (bs, 1H), 7.02 (bs, 1H), 7.06 (t, *J* = 8.8 Hz, 2H), 7.18 (dd, *J* = 1.7 Hz, *J* = 8.3 Hz, 1H), 7.28 (d, *J* = 2.5 Hz, 1H), 7.41 (d, *J* = 8.5 Hz, 1H), 7.50 (d, *J* = 1.7 Hz, 1H), 7.52 (dd, *J* = 5.3 Hz, *J* = 8.8 Hz, 2H), 7.71 (bs, 1H), 10.90 (bs, 1H); δ_C ($CDCl_3$, 125 MHz) 42.8 (CH_2), 110.1 (CH), 115.5 (CH), 118.4 (CH), 119.3 (CH), 119.8 (CH), 121.8 (C_q), 124.7 (CH), 125.5 (C_q), 128.7 (CH), 128.9 (CH), 135.1 (CH), 136.8 (C_q), 137.2 (C_q), 138.0 (C_q), 162.1 (CF); MS (ESI): *m/z* = 292 [M^++H].

1-((4-(4-Methoxyphenyl)naphthalen-1-yl)methyl)-*1H*-imidazole, 6.

Synthesised according to Method A1 using **6a** (0.38 g, 1.42 mmol) and CDI (1.84 g, 11.35 mmol); yield: 0.37 g (83 %); white solid: mp 192 °C; R_f = 0.25 (EtOAc / MeOH, 95:5); IR (ATR) υ (cm^{-1}) 3109 (w), 1609 (m), 1507 (s), 1460 (w), 1392 (w), 1284 (m), 1246 (s), 1176 (w), 1108 (w), 1076 (w), 1031 (m), 830 (m), 770 (m), 735 (w), 664 (w); δ_H ($CDCl_3$, 500 MHz) 3.89 (s, 3H), 5.61 (s, 2H), 6.97 (s, 1H), 7.04 (d, *J* = 8.6 Hz, 2H), 7.11 (s, 1H), 7.19 (d, *J* = 7.3 Hz, 1H), 7.36 (d, *J* = 7.3 Hz, 1H), 7.39 (d, *J* = 8.6 Hz, 2H), 7.46-7.49 (m, 1H), 7.53-7.56 (m, 1H), 7.61 (s, 1H), 7.93 (d, *J* = 8.4 Hz, 1H), 7.99 (d, *J* = 8.4 Hz, 1H); δ_C ($CDCl_3$, 125 MHz) 48.8, 53.4, 55.3, 113.8, 119.4, 122.5, 125.7, 126.1, 126.4, 126.7, 127.3, 129.6, 130.4, 131.0, 131.1, 132.2, 132.5, 137.5, 141.2, 159.1; (ESI): *m/z* = 315 [M^++H].

8-(1-(*1H*-Imidazol-1-yl)propyl)-5-(4-methoxyphenyl)quinoline, 8.

Synthesised according to Method A1 using **8a** (0.40 g, 1.36 mmol) and CDI (0.88 g, 5.45 mmol); yield: 0.24 g (51 %); white solid: mp 135 °C; R_f = 0.27 (EtOAc / MeOH, 95:5); IR (ATR) υ (cm^{-1}) 2967 (w), 1609 (m), 1515 (s), 1465 (w), 1284 (m), 1248 (s), 1176 (m), 1110 (w), 1074 (w), 1031 (m), 824 (s), 735 (w), 666 (m), 541 (w), 530 (w), 514 (w); δ_H ($CDCl_3$, 500 MHz) 1.06 (t, *J* = 7.3 Hz, 3H), 2.33-2.49 (m, 2H), 3.89 (s, 3H), 6.64-6.67 (m, 1H), 7.04 (d, *J* = 8.7 Hz, 2H), 7.06 (s, 1H), 7.13 (m, 1H), 7.35 (d, *J* = 8.7 Hz, 2H), 7.38 (dd, *J* = 4.1 Hz, *J* = 8.6 Hz, 1H), 7.43 (d, *J* = 7.5 Hz, 1H), 7.55 (d, *J*

= 7.5 Hz, 1H), 7.79 (s, 1H), 8.25 (dd, *J* = 1.7 Hz, *J* = 8.6 Hz, 1H), 8.94 (dd, *J* = 1.7 Hz, *J* = 4.1 Hz, 1H);δ_C ($CDCl_3$, 125 MHz) 11.4, 28.2, 55.4, 57.0, 114.0, 118.0, 121.2, 125.3, 126.8, 127.0, 128.9, 131.0, 131.4, 134.7, 137.1, 137.8, 140.3, 145.7, 149.4, 159.3; (ESI): *m/z* = 344 [M^++H].

8-(1-(*1H*-Imidazol-1-yl)propyl)-2-ethyl-5-(4-methoxyphenyl)quinoline, 10.

Synthesised according to Method A1 using **10a** (0.40 g, 1.25 mmol) and CDI (1.61 g, 9.96 mmol); yield: 0.27 g (59 %); white solid: mp 157 °C; R_f = 0.24 (EtOAc / MeOH, 95:5); IR (ATR) υ (cm^{-1}) 2969 (w), 1609 (s), 1576 (w), 1517 (s), 1498 (w), 1460 (w), 1285 (w), 1247 (s), 1177 (m), 1110 (w), 1072 (w), 1032 (m), 825 (w), 738 (m), 664 (m); δ_H ($CDCl_3$, 500 MHz) 1.08 (t, *J* = 7.3 Hz, 3H), 1.47 (t, *J* = 7.6 Hz, 3H), 2.37-2.51 (m, 2H), 3.06 (q, *J* = 7.6 Hz, *J* = 15.1 Hz, 2H), 3.91 (s, 3H), 6.69 (m, 1H), 7.05 (d, *J* = 8.7 Hz, 2H), 7.14 (s, 1H), 7.27 (d, *J* = 3.8 Hz, 1H), 7.35-7.39 (m, 3H), 7.55 (d, *J* = 7.5 Hz, 1H), 7.82 (s, 1H), 8.15 (d, *J* = 8.7 Hz, 1H); δ_C ($CDCl_3$, 125 MHz) 11.4, 13.2, 28.0, 31.9, 55.4, 57.1, 113.9, 117.9, 121.1, 125.1, 125.3, 125.8, 128.8, 131.0, 131.7, 134.7, 137.1, 137.2, 140.0, 145.2, 159.2, 162.6; (ESI): *m/z* = 372 [M^++H].

1-(1-(4-(Benzo*[b]*thiophen-5-yl)phenyl)propyl)-*1H*-imidazole, 12.

Synthesised according to Method A1 using **12a** (1.33 g, 4.94 mmol) and CDI (4.00 g, 24.7 mmol); yield: 0.51 g (32 %); white solid: mp 101-103 °C; R_f = 0.24 (DCM / MeOH, 20:1); IR (ATR) υ (cm^{-1}) 1496 (m), 1223 (m), 1073 (m), 805 (vs), 758 (s), 702 (s), 663 (s); δH ($CDCl_3$, 500 MHz) 0.98 (t, *J* = 7.3 Hz, 3H, CH_3), 2.28 (q, *J* = 7.3, 7.6 Hz, 2H, CH_2), 5.08 (t, *J* = 7.6 Hz, 1H, CH), 7.00 (s, 1H), 7.13 (s, 1H), 7.29 (d, *J* = 8.1 Hz, 2H), 7.39 (d, *J* = 5.4 Hz, 1H), 7.49 (d, *J* = 5.4 Hz, 1H), 7.55 (dd, *J* = 1.7, 8.4 Hz , 1H), 7.64 (dd, *J* = 1.7, 8.3 Hz, 2H), 7.73 (s, 1H), 7.93 (d, *J* = 8.4 Hz, 1H), 8.00 (s, 1H); δC ($CDCl_3$, 125 MHz) 11.1 (CH_3), 28.6 (CH_2), 63.3 (CH), 117.8, 120.0, 121.9, 122.8, 123.7, 124.0, 127.0, 127.2, 127.8, 128.7, 136.1, 136.7, 138.8, 139.1, 139.4, 140.2, 141.3; MS (ESI): m/z = 319 [M^++H].

5-(4-(1-(*1H*-Imidazol-1-yl)propyl)phenyl)-*1H*-indole, 13.

Synthesised according to Method A1 using **13a** (1.10 g, 4.38 mmol) and CDI (3.55 g, 21.88 mmol); yield: 0.33 g (25 %); white solid: mp 158-159 °C; R_f = 0.15 (DCM / MeOH, 20:1); IR (ATR) υ (cm^{-1}) 3143 (br), 1593 (w), 1471 (w), 1222 (w), 1075 (m), 891 (s), 805 (s), 772 (s), 739 (s), 660 (m), 574 (w), 539 (w); δH ($CDCl_3$, 500 MHz) 0.86 (t, *J* = 7.3, 3H, CH_3), 2.15 (q, *J* = 7.23, 7.6 Hz, 2H, CH_2), 4.93 (t, *J* = 7.6 Hz, 1H, CH), 6.50 (s, 1H), 6.90 (s, 1H), 7.04 (s, 1H), 7.13-7.17 (m, 3H), 7.30 (dd, *J* = 8.5, 9.5 Hz, 2H), 7.52 (d, *J* = 8.3 Hz, 2H), 7.67 (s, 1H), 7.74 (s, 1H), 8.89 (s, 1H); δC ($CDCl_3$, 125 MHz) 11.0 (CH_3), 28.4 (CH_2), 63.5 (CH), 102.6, 111.4, 119.0,121.4, 125.2, 127.7, 128.4, 130.8, 132.1, 135.6, 135.8, 137.3, 142.6; MS (ESI): m/z = 302 [M^++H].

1-(4-(Benzofuran-5-yl)benzyl)-*1H*-imidazole, 14.

Synthesised according to Method A1 using **14a** (0.50 g, 2.23 mmol) and CDI (1.81 g, 11.1 mmol); yield: 0.26 g (43 %); white solid: mp 127-129 °C; R_f = 0.22 (DCM / MeOH, 20:1); IR (ATR) υ (cm^{-1}) 3109 (w), 1511 (m), 1463 (m), 1439 (m), 1249 (m), 1130 (m), 1107 (m), 1083 (m), 1028 (s), 909 (m), 876 (m), 836 (m), 802 (vs), 779 (s), 748 (vs), 704 (m), 662 (vs), 631 (m), 523 (s); δH ($CDCl_3$, 500 MHz) 5.17 (s, 2H, CH_2), 6.82 (d, *J* = 3.0 Hz, 1H), 6.95 (s, 1H), 7.12 (s, 1H), 7.23 (d, *J* = 8.1 Hz, 2H),

7.49 (d, *J* = 5.4 Hz, 1H), 7.55~7.60 (m, 4H), 7.66 (d, *J* = 8.4 Hz, 1H), 7.76 (s, 1H); δC ($CDCl_3$, 125 MHz) 50.5 (CH_2), 119.7, 123.8, 127.7, 127.9, 129.9, 135.5, 137.4, 141.7,145.7; MS (ESI): m/z = 275 [M^++H].

1-(4-(Benzo*[b]*thiophen-5-yl)benzyl)-*1H*-imidazole, 15.

Synthesised according to Method A1 using **15a** (0.7 g, 2.91 mmol) and CDI (2.36 g, 14.6 mmol); yield: 0.18 g (21 %); white solid: mp 164-165 °C; R_f = 0.25 (DCM / MeOH, 20:1); IR (ATR) υ (cm^{-1}) 1434 (w), 908 (m), 807 (m), 759 (m), 708 (m), 663 (m); δH ($CDCl_3$, 500 MHz) 5.18 (s, 2H, CH_2), 6.95 (s, 1H), 7.12 (s, 1H), 7.25 (d, *J* = 8.1 Hz, 2H), 7.38 (d, *J* = 5.4 Hz, 1H), 7.49 (d, *J* = 5.4 Hz, 1H), 7.55 (d, *J* =8.4 Hz, 1H), 7.59 (s, 1H), 7.64 (d, *J* = 8.3 Hz, 2H), 7.93 (d, *J* = 8.4 Hz, 1H), 8.00 (s, 1H); δC ($CDCl_3$, 125 MHz) 50.5 (CH_2), 119.3, 121.93, 122.83, 123.7, 124.1, 127.3, 127.9, 129.9, 135.0, 139.1, 140.2, 141.4; MS (ESI): m/z = 291 [M^++H].

5-(4-((*1H*-Imidazol-1-yl)methyl)phenyl)-*1H*-indole, 16.

Synthesised according to Method A1 using **16a** (0.80 g, 3.58 mmol) and CDI (3.55 g, 21.88 mmol); yield: 0.24 g (25 %); white solid: mp 217-218 °C; R_f = 0.16 (DCM / MeOH, 20:1); IR (ATR) υ (cm^{-1}) 1509 (m), 1232 (m), 1099 (m), 1072 (m), 915 (m), 883 (m), 801 (m), 732 (s), 662 (m), 619 (m), 574 (s), 528 (m); δH ($CDCl_3$, 500 MHz) 5.16 (s, 2H, CH_2), 6.61-6.63 (m, 1H), 6.97 (s, 1H), 7.13 (s, 1H), 7.22 (d, *J* = 8.4 Hz, 2H), 7.25 (s, 1H), 7.43 (dd, *J* = 8.4, 9.4 Hz, 2H), 7.61 (s, 1H), 7.63 (d, *J* = 8.3 Hz, 2H), 7.84 (s, 1H), 8.31 (s, 1H); δC ($CDCl_3$, 125 MHz) 50.6 (CH_2), 103.0, 111.3, 119.2, 121.6, 127.6, 127.8, 128.4, 130.8, 132.4, 134.0, 135.5, 140.53, 142.63; MS (ESI): m/z = 274 [M^++H].

6-(4-((*1H*-Imidazol-1-yl)methyl)phenyl)benzo*[d]*thiazole, 17.

Synthesised according to Method A1 using **17a** (0.40 g, 1.66 mmol) and CDI (2.15 g, 13.26 mmol); yield: 0.29 g (70 %); white solid: mp 143 °C; R_f = 0.47 (EtOAc / MeOH, 95:5); IR (ATR) υ (cm^{-1}) 3370 (w), 2927 (w), 2856 (w), 1708 (w), 1506 (m), 1468 (m), 1441 (m), 1391 (m), 1284 (w), 1232 (m), 1108 (w), 1077 (m), 1030 (w), 886 (w), 813 (s), 739 (s), 697 (w), 663 (s); δ_H ($CDCl_3$, 500 MHz) 5.18 (s, 2H), 6.95 (s, 1H), 7.13 (s, 1H), 7.23 (d, *J* = 8.3 Hz, 2H), 7.64 (bs, 1H), 7.71 (dd, *J* = 1.8 Hz, *J* = 8.5 Hz, 1H), 8.12 (m, 1H), 8.18 (d, *J* = 8.5 Hz, 1H); δ_C ($CDCl_3$, 125 MHz) 50.6, 119.3, 120.1, 123.8, 125.8, 127.9, 128.0, 134.6, 135.3, 138.1, 140.6, 152.7, 154.3.

1-((4-(2,3-Dihydrobenzo*[b][*1,4]dioxin-6-yl)phenyl)methyl)-*1H*-imidazole, 18.

Synthesised according to Method A1 using **18a** (0.13 g, 0.54 mmol) and CDI (0.70 g, 4.30 mmol); yield: 0.06 g (43 %); white solid: mp 149 °C; R_f = 0.38 (EtOAc / MeOH, 95:5); IR (ATR) υ (cm^{-1}) 3112 (w), 3041 (w), 2930 (w), 2877 (w), 1728 (w), 1677 (w), 1588 (w), 1497 (s), 1309 (s), 1284 (m), 1246 (m), 1068 (s), 897 (m), 876 (w), 807 (m), 748 (m), 699 (w), 662 (w), 530 (w); δ_H ($CDCl_3$ + CD_3OD, 500 MHz) 5.13 (s, 2H), 6.88 (d, *J* = 8.3 Hz, 1H), 6.96 (bs, 1H), 7.00-7.04 (m, 3H), 7.18 (d, *J* = 8.0 Hz, 2H), 7.36 (s, 1H), 7.48 (d, *J* = 8.0 Hz, 2H), 7.64 (s, 1H); δ_C ($CDCl_3$ + CD_3OD, 125 MHz) 17.8, 29.8, 31.0, 51.1, 64.8, 64.8, 116.0, 117.9, 120.1, 120.3, 127.6, 128.2, 128.9, 134.1, 134.5, 137.6, 141.1, 143.8, 144.1; (ESI): *m/z* = 293 [M^++H].

1-(1-(4-(6-Methoxynaphthalen-2-yl)phenyl)propyl)-*1H*-imidazole, 19.

Synthesised according to Method A1 using **19a** (0.75 g, 1.71 mmol) and CDI (1.50 g, 9.30 mmol); yield: 0.23 g (39 %); white solid: mp 136-138 °C; R_f = 0.27 (DCM / MeOH, 95:5); IR (ATR) υ (cm^{-1}), 2936 (w), 1676 (s), 1602 (m), 1502 (m), 1462 (m), 1200 (s), 1021 (m), 844 (s), 814 (s), 665 (s), 532 (m); δ_H ($CDCl_3$, 500 MHz) 0.98 (t, *J*=7.25 Hz, 3H, CH_3), 2.27-2.30 (m, 2H, CH_2), 3.94 (s, 3H, OCH_3), 5.06 (t, *J*=7.61 Hz, 1H, CH), 6.99 (s, 1H, Im-H5), 7.11 (s, 1H, Im-H4), 7.16-7.19 (m, 2H, Aromat), 7.28 (d, *J*=8.19 Hz, 2H, Aromat), 7.65 (s, 1H, Im-H2), 7.66-7.68 (m, 3H, Aromat), 7.80 (t, *J*=8.51 Hz, 2H, Aromat), 7.95 (d, *J*=1.26 Hz, 1H, Aromat); δ_C ($CDCl_3$, 125 MHz) 11.1 (CH_3), 28.6 (CH_2), 55.3 (CH), 63.0 (OCH_3), 105.6 (C-5´), 117.7 (Im-C4), 119.2 (C-7´), 125.6, 125.7 (Im-C5, C-3´), 127.0, 127.3 (C-1´, C-4´), 127.6 (C-3, C-5), 129.1 (C-2, C-6), 129.7 (C-8), 133.9 (C-2´), 135.4 (C-4), 139.0 (C-1), 141.0 (Im-C2), 157.9 (C-6´); MS (ESI): *m/z* = 343 [M^+-H].

1-(1-(4-(Naphthalen-2-yl)phenyl)propyl-*1H*-imidazole, 20.

Synthesised according to Method A1 using **20a** (0.50 g, 1.9 mmol) and CDI (1.50 g, 9.30 mmol); yield: 0.23 g (39 %); white solid: mp 136-138 °C; R_f = 0.29 (DCM / MeOH, 95:5); IR (ATR) υ (cm^{-1}), 1687 (m), 1499 (s), 1223 (m), 1072 (m), 1015 (m), 809 (vs), 758 (s), 733 (s), 662 (s); δ_H ($CDCl_3$, 500 MHz) 0.98 (t, *J*=7.25 Hz, 3H, CH_3), 2.24-2.33 (m, 2H, CH_2), 5.08 (t, *J*=7.56 Hz, 1H, CH), 7.00 (t, *J*=1.26 Hz, 1H, Im-H5), 7.11 (t, *J*=1.26 Hz, 1H, Im-H4), 7.30 (d, *J*=7.88 Hz, 2H, aromat), 7.47-7.53 (m, 2H, aromat), 7.65 (s, 1H, Im-H2), 7.69-7.72 (m, 3H, aromat), 7.85-7.92 (m, 3H, aromat), 8.01 (d, *J*=1.57 Hz, 1H, aromat); δ_C ($CDCl_3$, 125 MHz) 11.1 (CH_3), 28.6 (CH_2), 63.1 (CH), 117.7 (Im-C4), 125.3 (C-3´), 125.8, 126.1, 126.4 (Im-C5, C-7´, C-8´), 127.1 (C-1´), 127.6, 127.8, 128.1, 128.5 (C-3, C-5, C-5´, C-8´), 129.6 (C-4´), 132.7, 132.6 (C-2, C-6), 136.4 (C-4, C-2´), 137.6 (C-1), 139.4 (Im-C2); MS (ESI): *m/z* = 313 [M^++H].

1-(3-(4-(6-(tert-Butyldimethylsilyloxy)naphthalen-2-yl)phenyl)pentan-3-yl)-1*H*-imidazole, 21a. Synthesised according to Method A1 using **21b** (1.00 g, 2.5 mmol) and CDI (2.06 g, 12.7 mmol); brown oil; the crude product was directly used in the next step without further purification and analysis.

Method B: Ether cleavage with BBr_3.

To a solution of the corresponding ether (1 eq) in DCM (5 mL / mmol) at -78 °C was added 1 M borontribromide in DCM (5 eq). The resulting mixture was stirred at rt for 16 hours. Then water (25 mL) was added and the emulsion was stirred for further 30 minutes. The resulting mixture was extracted with ethyl acetate (3 x 25 mL). The combined organic phases were washed with brine, dried over Na_2SO_4 and evaporated under reduced pressure. Then the desired product was purified by chromatography on silica gel.

4-(2-((*1H*-Imidazol-1-yl)methyl)benzo*[b]*thiophen-5-yl)phenol, 2.

Synthesised according to Method B using **1** (0.15 g, 0.47 mmol) and BBr_3 (2.35 mL, 2.34 mmol); yield: 0.05 g (32 %); white solid: mp 211 °C; R_f = 0.42 (DCM / MeOH, 95:5); IR (ATR) υ (cm^{-1}) 3349 (w), 2959 (w), 1733 (w), 1609 (w), 1515 (s), 1448 (w), 1277 (s), 1242 (w), 1231 (w), 1194 (w), 1108 (m), 1030 (w), 951 (w), 885 (w), 809 (s), 749 (s), 658 (m), 547 (w); δ_H ($CDCl_3$ + CD_3OD, 500 MHz)

5.43 (s, 2H), 6.88 (d, *J* = 8.6 Hz, 2H), 7.02 (bs, 1H), 7.11 (bs, 1H), 7.25 (s, 1H), 7.44-7.52 (m, 3H), 7.72 (bs, 1H), 7.76 (d, *J* = 8.4 Hz, 1H), 7.85 (s, 1H); δ_C ($CDCl_3$ + CD_3OD, 125 MHz) 116.3, 122.0, 123.1, 124.4, 124.8, 128.9, 133.1, 138.9, 138.9, 140.3, 140.6, 157.2.

4-(4-((*1H*-Imidazol-1-yl)methyl)naphthalen-1-yl)phenol, 7.

Synthesised according to Method B using **6** (0.15 g, 0.48 mmol) and BBr_3 (1.90 mL, 1.90 mmol); yield: 0.08 g (52 %); white solid: mp 197 °C; R_f = 0.42 (DCM / MeOH, 95:5); IR (ATR) υ (cm^{-1}) 3600-2900 (w), 1610 (w), 1509 (m), 1437 (w), 1391 (w), 1264 (s), 1214 (w), 1172 (w), 1091 (m), 951 (w), 834 (s), 770 (w), 734 (s), 654 (w), 574 (w); δ_H ($CDCl_3$ + CD_3OD, 500 MHz) 6.89 (d, *J* = 8.4 Hz, 2H), 7.04 (s, 1H), 7.15 (s, 1H), 7.21 (d, *J* = 8.4 Hz, 2H), 7.28-7.30 (m, 1H), 7.31 (s, 1H), 7.36-7.39 (m, 1H), 7.44-7.47 (m, 1H), 7.82 (d, *J* = 8.4 Hz, 1H), 7.93 (d, *J* = 8.4 Hz, 1H), 7.29 (bs, 1H); δ_C ($CDCl_3$ + CD_3OD, 125 MHz) 115.3, 122.4, 126.3, 126.4, 127.2, 127.6, 131.1, 131.3, 132.5, 156.6; (ESI): *m/z* = 301 [M^++H].

4-(8-(1-(*1H*-Imidazol-1-yl)propyl)quinolin-5-yl)phenol, 9.

Synthesised according to Method B using **8** (0.10 g, 0.29 mmol) and BBr_3 (1.46 mL, 1.46 mmol); yield: 0.06 g (63 %); white solid: mp 174 °C; R_f = 0.42 (DCM / MeOH, 95:5); IR (ATR) υ (cm^{-1}) 3500-2600 (w), 1610 (m), 1515 (s), 1458 (w), 1397 (w), 1269 (s), 1227 (w), 1172 (w), 1108 (w), 1089 (w), 826 (s), 797 (w), 736 (s), 669 (w); δ_H ($CDCl_3$ + CD_3OD, 500 MHz) 0.95 (t, *J* = 7.2 Hz, 3H), 2.30-2.41 (m, 2H), 6.58 (m, 1H), 6.91 (d, J = 8.5 Hz, 2H), 6.07 (bs, 1H), 7.14 (bs, 1H), 7.18 (d, *J* = 8.5 Hz, 2H), 7.28-7.32 (m, 1H), 7.37 (d, *J* = 7.5 Hz, 1H), 7.57 (d, *J* = 7.5 Hz, 1H), 7.99 (bs, 1H), 8.22 (d, *J* = 8.5 Hz, 1H), 8.81 (m, 1H); δ_C ($CDCl_3$ + CD_3OD, 125 MHz) 11.3, 27.8, 57.8, 60.7, 115.5, 115.6, 119.0, 121.4, 125.8, 125.9, 126.8, 127.2, 127.3, 130.0, 131.1, 131.1, 135.3, 135.9, 137.3, 141.4, 145.7, 149.6, 157.1; (ESI): *m/z* = 330 [M^++H].

4-(8-(1-(*1H*-Imidazol-1-yl)propyl)-2-ethylquinolin-5-yl)phenol, 11.

Synthesised according to Method B using **10** (0.15 g, 0.40 mmol) and BBr_3 (2.00 mL, 2.00 mmol); yield: 0.09 g (58 %); white solid: mp 142 °C; R_f = 0.69 (DCM / MeOH, 95:5); IR (ATR) υ (cm^{-1}) 3600-2800 (w), 2968 (w), 2932 (w), 1733 (s), 1608 (s), 1579 (w), 1518 (s), 1457 (w), 1398 (w), 1269 (s), 1225 (m), 1172 (m), 1108 (w), 951 (w), 824 (s), 734 (m), 659 (w); δ_H ($CDCl_3$ + CD_3OD, 500 MHz) 0.94 (t, *J* = 7.2 Hz, 3H), 1.30 (t, *J* = 7.5 Hz, 3H), 2.29-2.43 (m, 2H), 2.89-2.93 (m, 2H), 6.57-6.60 (m, 1H), 6.88 (d, *J* = 8.1 Hz, 2H), 6.99 (s, 1H), 7.12 (s, 1H), 7.15-7.18 (m, 3H), 7.29 (d, *J* = 7.4 Hz, 1H), 7.53 (d, *J* = 7.4 Hz, 1H), 8.08 (d, *J* = 8.7 Hz, 1H), 8.13 (s, 1H); δ_C ($CDCl_3$ + CD_3OD, 125 MHz) 11.0, 13.0, 27.1, 31.6, 58.1, 115.2, 119.1, 121.0, 125.2, 125.33, 125.34, 126.2, 130.2, 130.8, 134.2, 134.8, 137.5, 141.0, 145.0, 156.4, 162.9; (ESI): *m/z* = 358 [M^++H].

Method C: Suzuki-Coupling.

The corresponding brominated aromatic compound (1 eq) was dissolved in toluene (7 mL / mmol), an aqueous 2.0 M Na_2CO_3 solution (3.2 mL / mmol) and an ethanolic solution (3.2 mL / mmol) of the corresponding boronic acid (1.5-2.0 eq) were added. The mixture was deoxygenated under reduced pressure and flushed with nitrogen. After repeating this cycle several times $Pd(PPh_3)_4$ (4 mol%) was

added and the resulting suspension was heated under reflux for 8 h. After cooling ethyl acetate (10 mL) and water (10 mL) were added and the organic phase was separated. The water phase was extracted with ethyl acetate (2 x 10 mL). The combined organic phases were washed with brine, dried over Na_2SO_4, filtered over a short plug of celite® and evaporated under reduced pressure. The compounds were purified by flash chromatography on silica gel.

6-(4-Fluorophenyl)-*1H*-indole-3-carbaldehyde, 3b.

Synthesised according to Method C1 using 6-bromo-1*H*-indole-3-carbaldehyde (0.45 g, 2.00 mmol) and 4-fluorophenylboronic acid (0.57 g, 4.05 mmol); yield: 0.35 g (73 %); yellow oil; R_f = 0.57 (petrolether / EtOAc, 1:2); IR (ATR) υ (cm^{-1}) 3228 (m), 2806 (w), 1646 (s), 1618 (m), 1518 (m), 1386 (m), 1198 (m), 1110 (s), 1083 (s), 811 (s), 670 (s), 593 (m), 517 (s); δ_H ($CDCl_3$, 500 MHz) 6.84 (t, *J* = 8.8 Hz, 2H), 7.15 (dd, *J* = 1.6 Hz, 8.2 Hz, 1H), 7.30 (dd, *J* = 5.4 Hz, 8.8 Hz, 2H), 7.33 (m, 1H), 7.63 (d, *J* = 3.2 Hz, 1H), 7.96 (d, *J* = 8.2 Hz, 1H), 9.71 (s, 1H), 11.42 (bs, 1H); δ_C ($CDCl_3$, 125 MHz) 109.7 (CH), 114.8 (CH), 118.0 (C_q), 121.0 (C_q), 121.1 (CH), 123.0 (C_q), 128.1 (CH), 135.3 (C_q), 136.8 (CH), 137.1 (C_q), 137.3 (C_q), 161.4 (CF), 184.2 (CH); MS (ESI): m/z = 238 [M^+-H].

2-((*1H*-Imidazol-1-yl)methyl)-6-(4-fluorophenyl)quinoline, 4.

Synthesised according to Method C1 using **4a** (0.24 g, 0.84 mmol) and 4-fluorophenylboronic acid (0.23 g, 1.68 mmol); yield: 0.23 g (92 %); **y**ellow solid, R_f = 0.15 (EtOAc/MeOH 9/1); IR (ATR) υ (cm^{-1}) 3125 (w), 3094 (w), 2926 (w), 1602 (m), 1515 (m), 1498 (m), 1431 (m), 1224 (s), 824 (s), 771 (m), 657 (m); δ_H ($CDCl_3$, 500 MHz) 5.43 (s, 2H), 7.04 (t, *J* = 1.3 Hz, 1H), 7.08 (d, *J* = 8.5 Hz, 1H), 7.15 (bs, 1H), 7.18 (t, *J* = 8.5, 2H), 7.66 (m, 2H), 7.69 (bs, 1H), 7.93 (d, *J* = 2.2 Hz, 1H), 7.96 (dd, *J* = 2.2, 8.5 Hz, 1H), 8.12 (d, *J* = 8.8 Hz, 1H), 8.16 (d, *J* = 8.5 Hz, 1H); δ_C ($CDCl_3$, 125 MHz) 53.2 (CH_2), 115.9 (d, $^2J_{CF}$ = 22 Hz, CH), 119.1 (CH), 119.6 (CH), 125.1 (CH), 127.6 (CH), 128.3 (C_q), 128.4 (CH), 129.0 (d,³ *J* = 7.7 Hz, CH), 129.6 (CH), 129.7 (CH), 130.2 (CH), 136.2 (d,$^4J_{CF}$ = 2.9 Hz, C_q), 137.7 (CH), 137.8 (CH), 138.8 (C_q), 146.9 (C_q), 156.2 (C_q), 162.8 (d, $^1J_{CF}$ = 248.0 Hz, CF); MS (ESI): *m/z* = 304 [M^++H].

2-((*1H*-Imidazol-1-yl)methyl)-6-(thiophen-3-yl)quinoline, 5.

Synthesised according to Method C1 using **5a** (0.24 g, 0.84 mmol) and 3-thiophenylboronic acid (0.22 g, 1.68 mmol); yield: 0.22 g (88 %); yellow solid; R_f = 0.11 (EtOAc/MeOH 9/1); IR (ATR) υ (cm^{-1}) 3104 (w), 2963 (w), 2926 (m), 1597 (m), 1506 (m), 1318 (m), 1068 (m), 828 (s), 782 (s), 753 (s), 660 (s); δ_H ($CDCl_3$, 500 MHz) 5.43 (s, 2H), 7.04 (t, *J* = 1.3 Hz, 1H), 7.06 (d, *J* = 8.5 Hz, 1H), 7.15 (t, *J* = 0.9 Hz, 1H), 7.46 (dd, *J* = 2.8, 5.0 Hz, 1H), 7.52 (dd, *J* = 1.6, 5.0 Hz, 1H), 7.62 (dd, *J* = 1.3, 2.8 Hz, 1H), 7.69 (bs, 1H), 7.99 (d, *J* = 1.9 Hz, 1H), 8.02 (dd, *J* = 2.2, 8.8 Hz, 1H), 8.09 (d, *J* = 8.8 Hz, 1H), 8.14 (d, *J* = 8.5 Hz, 1H); δ_C ($CDCl_3$, 125 MHz) 53.2 (CH_2), 119.1 (CH), 119.6 (CH), 121.5 (CH), 124.2 (CH), 126.3 (CH), 126.8 (CH), 127.7 (C_q), 129.4 (CH), 129.6 (CH), 130.2 (CH), 134.4 (C_q), 137.6 (CH), 137.8 (CH), 141.2 (C_q), 146.9 (C_q), 155.9 (C_q); MS (ESI): *m/z* = 292 [M^++H].

(4-(4-Methoxyphenyl)naphthalen-1-yl)methanol, 6a.

Synthesised according to Method C2 using **6b** (0.40 g, 1.69 mmol) and 4-methoxyphenylboronic acid (0.39 g, 2.53 mmol); yield: 0.42 g (94 %); white solid; R_f = 0.57 (petrolether / EtOAc, 1:2); δ_H ($CDCl_3$, 500 MHz) 3.90 (s, 3H), 5.20 (s, 2H), 7.04 (d, *J* = 8.7 Hz, 2H), 7.38 (d, *J* = 7.2 Hz, 1H), 7.40 (d, *J* = 8.7 Hz, 2H), 7.45-7.48 (m, 1H), 7.56 (d, *J* = 7.2 Hz, 2H), 7.96 (d, *J* = 8.4 Hz, 1H), 8.20 (d, *J* = 8.4 Hz, 1H); δ_C ($CDCl_3$, 125 MHz) 55.4, 63.8, 113.7, 123.9, 125.0, 125.9, 126.2, 126.4, 126.9, 131.1, 131.5, 132.2, 133.0, 135.4, 140.5, 159.0.

5-(4-Methoxyphenyl)quinoline-8-carbaldehyde, 8b.

Synthesised according to Method C2 using 6-bromo-1*H*-indole-3-carbaldehyde (3.00 g, 12.71 mmol) and 4-methoxyphenylboronic acid (2.90 g, 19.06 mmol); yield: 2.75 g (82 %); white solid; R_f = 0.63 (petrolether / EtOAc, 1:2); δ_H ($CDCl_3$, 500 MHz) 7.06 (d, *J* = 8.7 Hz, 2H), 7.41 (d, *J* = 8.7 Hz, 2H), 7.45 (d, *J* = 4.1 Hz, *J* = 8.6 Hz, 1H), 7.61 (d, *J* = 7.5 Hz, 1H), 8.33-8.34 (m, 2H), 9.03-9.04 (m, 1H), 11.48 (s, 1H); δ_C ($CDCl_3$, 125 MHz) 55.4, 114.1, 121.5, 121.8, 126.2, 126.7, 126.9, 128.8, 129.3, 130.5, 130.9, 131.0, 134.2, 134.8, 136.3, 146.7, 148.1, 150.9, 151.3, 159.8, 192.6.

4-(Benzo*[b]*thiophen-5-yl)benzaldehyde, 12b.

Synthesised according to Method C1 using 5-bromobenzo*[b]*thiophene (1.90 g, 8.92 mmol) and 4-formylphenylboronic acid (1.74 g, 11.6 mmol); yield: 1.71 g (80 %); white solid; R_f = 0.35 (petrolether / EtOAc, 5:1); δH ($CDCl_3$, 500 MHz) 7.41 (d, *J* = 5.4 Hz, 1H), 7.51 (d, *J* = 5.4 Hz, 1H), 7.61 (d, *J* = 8.4 Hz, 1H), 7.82 (d, *J* = 7.9 Hz, 2H), 7.97 (d, *J* = 8.2 Hz, 3H), 8.07 (s, 1H), 10.07 (s, 1H, CHO); δC ($CDCl_3$, 125 MHz) 122.3, 123.0, 123.6, 124.0, 127.5, 127.8, 130.3, 135.0, 126.1, 139.9, 140.2, 147.3, 191.9.

4-(*1H*-Indol-5-yl)benzaldehyde, 13b.

Synthesised according to Method C1 using 5-bromo-*1H*-indole (0.10 g, 0.51 mmol) and 4-formylphenylboronic acid (0.1 g, 0.66 mmol); yield: 0.07 g (65 %); white solid; R_f = 0.25 (petrolether / EtOAc, 5:1); δH ($CDCl_3$, 500 MHz) 6.64 (t, *J* = 2.8 Hz, 1H), 7.27 (t, *J* = 3.1 Hz, 1H), 7.48 (s, 1H), 7.74 (dd, *J* = 1.8, 8.5 Hz, 2H), 7.92 (s, 1H), 8.04 (dd, *J* = 1.8, 8.5 Hz, 2H), 8.29 (s, br, 1H), 10.05 (s, 1H, CHO); δC ($CDCl_3$, 125 MHz) 103.2, 111.4, 119.6, 121.7, 125.1, 127.2, 128.4, 128.5, 132.0, 135.8, 147.0, 192.1.

4-(Benzofuran-5-yl)benzaldehyde, 14b.

Synthesised according to Method C1 using 5-bromobenzofuran (1.90 g, 9.64 mmol) and 4-formylphenylboronic acid (1.88 g, 12.5 mmol); yield: 0.53 g (25 %); yellow oil; R_f = 0.33 (petrolether / EtOAc, 5:1); δH ($CDCl_3$, 500 MHz) 6.83-6.84 (m, 1H), 7.54-7.60 (m, 2H), 7.68 (d, *J* = 2.2 Hz, 1H), 7.76 (d, *J* = 8.2 Hz, 2H), 7.84 (d, *J* = 1.6 Hz, 1H), 7.94 (dd, *J* = 1.8, 8.4 Hz, 2H), 10.05 (s, 1H, CHO); δC ($CDCl_3$, 125 MHz) 106.8, 111.8, 120.1, 123.9, 127.8, 130.2, 134.8, 145.9, 147.6, 155.0, 191.9.

(4-(Benzo*[d]*thiazol-6-yl)phenyl)methanol, 17a.

Synthesised according to Method C1 using 6-bromobenzo*[d]*thiazole (0.50 g, 2.34 mmol) and 4-(hydroxymethyl)phenylboronic acid (0.53 g, 3.50 mmol); yield: 0.50 g (89 %); white solid; R_f = 0.26 (petrolether / EtOAc, 1:2); δ_H ($CDCl_3$, 500 MHz) 4.77 (s, 2H), 7.48 (d, *J* = 8.4 Hz, 2H), 7.64-7.68 (m,

3H), 7.74 (dd, *J* = 1.8 Hz, *J* = 8.5 Hz, 1H), 8.15 (s, 1H), 8.20 (d, *J* = 8.5 Hz, 1H), 9.03 (s, 1H); δ_C ($CDCl_3$, 125 MHz) 64.9, 120.1, 123.6, 125.9, 127.6, 127.6, 128.5, 128.6, 132.0, 132.0, 132.1, 132.1, 134.5, 138.8, 139.7, 140.4.

(4-(2,3-Dihydrobenzo*[b]*[1,4]dioxin-6-yl)phenyl)methanol, 18a.

Synthesised according to Method C1 using 6-bromo-2,3-dihydrobenzo*[b]*[1,4]dioxine (0.50 g, 2.33 mmol) and 4-(hydroxymethyl)phenylboronic acid (0.53 g, 3.49 mmol); yield: 0.15 g (27 %); white solid; R_f = 0.62 (petrolether / EtOAc, 1:2); δ_H ($CDCl_3$, 500 MHz) 4.29 (s, 1H), 4.71 (s, 1H), 6.93 (d, *J* = 8.3 Hz, 1H), 7.08 (dd, *J* = 2.2 Hz, *J* = 8.3 Hz, 1H), 7.12 (d, *J* = 2.2 Hz, 1H), 7.40 (d, *J* = 8.3 Hz, 2H), 7.53 (d, *J* = 8.3 Hz, 2H); δ_C ($CDCl_3$, 125 MHz) 64.4, 64.4, 65.1, 115.8, 117.5, 120.1, 126.9, 127.4, 134.4, 139.4, 140.0, 143.2, 143.7.

Method D: Reduction with $NaBH_4$.

To an ice-cooled solution of the corresponding aldehyde or ketone (1 eq) in methanol (5 mL / mmol) was added $NaBH_4$ (2 eq). Then the resulting mixture was heated to reflux for 30 minutes. After cooling to ambient temperature, the solvent was distilled off under reduced pressure. Then water (10 mL) was added, and the resulting mixture was extracted with ethyl acetate (3 x 10 mL). The combined organic phases were washed with brine, dried over $MgSO_4$ and evaporated under reduced pressure. Then the desired product was purified by chromatography on silica gel.

(6-(4-Fluorophenyl)-*1H*-indol-3-yl)-methanol, 3a.

Synthesised according to Method D using **3b** (0.30 g, 1.25 mmol) and $NaBH_4$ (0.86 g, 2.26 mmol); yield: 0.27 g (89 %); R_f = 0.55 (PE / EtOAc, 1:2); the crude product was directly used in the next step without further purification and analysis.

(4-(Benzo*[b]*thiophen-5-yl)phenyl)methanol, 14a.

Synthesised according to Method D using **14b** (0.30 g, 1.26 mmol) and $NaBH_4$ (0.10 g, 2.52 mmol); yield: 0.28 g (92 %); R_f = 0.30 (PE / EtOAc, 2:1); the crude product was directly used in the next step without further purification and analysis.

1-(4-(Benzo*[b]*thiophen-5-yl)phenyl)propan-1-ol, 15a.

Synthesised according to Method D using **15b** (0.30 g, 1.36 mmol) and $NaBH_4$ (0.11 g, 2.71 mmol); yield: 0.28 g (91 %); R_f = 0.30 (PE / EtOAc, 2:1); the crude product was directly used in the next step without further purification and analysis.

1-(4-(6-Methoxynaphthalen-2-yl)phenyl)propan-1-ol, 19a.

Synthesised according to Method D using **19b** (1.93 g, 6.40 mmol) and $NaBH_4$ (0.48 g, 12.8 mmol); yield: 0.711 g (38 %); R_f = 0.59 (DCM / MeOH, 98:2); the crude product was directly used in the next step without further purification and analysis.

1-(4-(Naphthalen-2-yl)phenyl)propan-1-ol, 20a.

Synthesised according to Method D using **20b** (1.80 g, 6.40 mmol) and $NaBH_4$ (0.48 g, 12.8 mmol); yield: 1.34 g (80 %); R_f = 0.62(DCM / MeOH, 98:2); the crude product was directly used in the next step without further purification and analysis.

6-Bromo-2-(bromomethyl)quinoline 4b.

6-Bromo-2-methylquinoline (3.00 g, 13.5 mmol) was dissolved in 40 mL of dry carbon tetrachloride. To this solution was added NBS (2.63 g, 14.8 mmol) and dibenzoyl peroxide (0.16 g, 0.70 mmol) and the mixture was refluxed over night. After cooling, the succinimide was removed by filtration and the filtrate was concentrated under vacuum. The crude product was further purified by flash column chromatography on silica gel using petroleum ether / EtOAc (95 : 5) as eluent; yield: 1.71 g (42%); lachrymatory lilac oil; R_f = 0.46 (hexane / EtOAc 95 : 5); IR (ATR) υ (cm^{-1}) 3054 (w), 3038 (w), 2928 (w), 2855 (w), 1589 (m), 1484 (s), 1373 (m), 1304 (m), 1200 (s), 1190 (s), 1060 (s), 899 (s), 830 (s), 792 (s), 775 (m), 735 (s), 694 (s), 597 (s), 550 (s); δ_H ($CDCl_3$, 500 MHz) 4.68 (s, 2H), 7.59 (d, *J* = 8.5 Hz, 1H), 7.79 (dd, *J* = 2.1, 9.1 Hz, 1H), 7.93 (d, *J* = 8.8 Hz, 1H), 7.98 (d, *J* = 2.1 Hz, 1H), 8.08 (d, *J* = 8.5 Hz, 1H); δ_C ($CDCl_3$, 125 MHz) 34.0 (CH_2), 121.0 (CH), 122.0 (CH), 128.4 (C_q), 129.6 (CH), 131.0 (CH), 133.4 (CH), 136.2 (C_q), 157.4 (C_q), 157.9 (C_q); MS (ESI): m/z =302 [M^+ + H].

2-(*1H*-Imidazol-1-ymethyl)-6-bromoquinoline 4a.

The α-brominated compound **4b** (1.89 g, 6.28 mmol), imidazole (0.86 g, 12.55 mmol), anhydrous K_2CO_3 (1.29 g, 9.42 mmol) and a catalytical amount of 18-crown-6 in dry acetonitrile were heated under reflux over night. After the solution was cooled down, the solvent was removed under reduced pressure. The residue was dissolved with water (10 mL/eq) and extracted three times with CH_2Cl_2 (15 mL/eq). The combined organic extracts were washed with brine, dried over anhydrous Na_2SO_4, filtered, and evaporated. The crude material was purified by flash chromatography on silica-gel, using 5% MeOH in CH_2Cl_2; yield: 1.1 g (61%); R_f = 0.23 (EtOAc / MeOH 9 : 1); IR (ATR) υ (cm^{-1}) 3098 (w), 1593 (m), 1488 (s), 1291 (m), 1235 (m), 1086 (m), 1030 (m), 824 (s), 733 (s), 658 (s); δ_H ($CDCl_3$, 500 MHz) 5.39 (s, 2H), 7.01 (t, *J* = 1.3 Hz, 1H), 7.06 (d, *J* = 8.5 Hz, 1H), 7.13 (t, *J* = 1.3 Hz, 1H), 7.66 (s, 1H), 7.79 (dd, *J* = 2.2, *J* = 9.1 Hz, 1H), 7.91 (d, *J* = 9.1 Hz, 1H), 7.96 (d, *J* = 2.2 Hz, 1H), 8.03 (d, *J* = 8.5 Hz, 1H)); δ_C ($CDCl_3$, 125 MHz) 55.0 (CH_2), 119.5 (CH), 119.5 (CH), 120.9 (CH), 129.6 (C_q), 130.2 (CH), 130.8 (CH), 133.6 (CH), 136.6 (CH), 137.7 (C_q), 146.2 (C_q), 156.6 (C_q); MS (ESI): m/z = 289 [M^+ + H].

4-Bromo-1-naphthoic acid, 6c.

4-Bromo-1-methyl naphthalene (20.0 g, 88.65 mmol) was preheated to 90 °C in a water / pyridine 1 : 1 mixture (170 mL) and $KMnO_4$ (57.2 g, 354.6 mmol) was added over a period of 3 h every 30 in in equal portions. yield: 1.60 g (7 %); R_f = 0.37 (PE / EtOAc, 2:1); δ_H ($CDCl_3$ + CD_3OD, 500 MHz) 7.56-7.61 (m, 2H), 7.78 (d, *J* = 7.9 Hz, 1H), 8.00 (d, *J* = 7.9 Hz, 1H), 8.26-8.28 (m, 1H), 8.91-8.93 (m, 1H); δ_C ($CDCl_3$ + CD_3OD, 125 MHz) 126.5, 127.6, 127.7, 128.4, 128.7, 129.1, 130.5, 132.3, 132.6.

(4-Bromonaphthalen-1-yl)methanol, 6b.

To a solution of **6c** (0.60 g, 2.39 mmol) in THF (10 mL) $LiAlH_4$ (0.91 g, 3.90 mmol) in THF (5 mL) was added slowly and heated to reflux overnight. yield: 1.65 g (83 %); R_f = 0.74 (PE / EtOAc, 2:1); δ_H ($CDCl_3$, 500 MHz) 5.12 (s, 2H), 7.37 (d, *J* = 7.6 Hz, 1H), 7.58-7.64 (m, 2H), 7.76 (d, *J* = 7.6

Hz, 1H), 8.11 (d, *J* = 7.8 Hz, 1H), 8.31 (d, *J* = 7.8 Hz, 1H); δ_C ($CDCl_3$, 125 MHz) 123.2, 124.0, 125.5, 127.1, 127.2, 127.9, 129.4, 132.1, 132.3, 136.3.

Method E: Grignard reaction.

Under exclusion of air and moisture a 1.0 M EtMgBr (1.2 eq) solution in THF was added dropwise to a solution of the aldehyde or ketone (1 eq) in THF (12 mL / mmol). The mixture was stirred over night at rt. Then ethyl acetate (10 mL) and water (10 mL) were added and the organic phase was separated. The organic phase was extracted with water and brine, dried over Na_2SO_4, and evaporated under reduced pressure. The crude products were purified by flash chromatography on silica gel.

1-(5-(4-Methoxyphenyl)quinolin-8-yl)propan-1-ol, 8a.

Synthesised according to Method E using **8b** (1.00 g, 3.80 mmol) and $NaBH_4$ (15.20 mL, 15.20 mmol); yield: 0.47 g (43 %); R_f = 0.47 (PE / EtOAc, 2:1); δ_H ($CDCl_3$, 500 MHz) 1.05 (t, *J* = 7.4 Hz, 3H), 1.97-2.20 (m, 2H), 3.90 (s, 3H), 5.04-5.07 (m, 1H), 7.04 (d, *J* = 8.6 Hz, 2H), 7.35-7.38 (m, 3H), 7.43 (d, *J* = 7.3 Hz, 1H), 7.56 (d, *J* = 7.3 Hz, 1H), 8.30 (d, *J* = 8.6 Hz, 1H), 8.82-8.83 (m, 1H); δ_C ($CDCl_3$, 125 MHz) 11.0, 31.9, 55.4, 113.9, 120.6, 126.8, 127.1, 127.4, 131.1, 131.5, 135.4, 139.2, 139.5, 146.8, 147.9, 150.3.

1-(2-Ethyl-5-(4-methoxyphenyl)quinolin-8-yl)propan-1-ol, 10a.

Synthesised according to Method E using **8b** (1.00 g, 3.80 mmol) and $NaBH_4$ (15.20 mL, 15.20 mmol); yield: 0.43 g (39 %); R_f = 0.52 (PE / EtOAc, 2:1); δ_H ($CDCl_3$, 500 MHz) 1.03 (t, *J* = 7.4 Hz, 3H), 1.41 (t, *J* = 7.6 Hz, 3H), 2.02-2.19 (m, 2H), 2.98-3.03 (m, 2H), 3.89 (s, 3H), 4.95-4.98 (m, 1H), 7.03 (d, *J* = 8.6 Hz, 2H), 7.24 (d, *J* = 8.8 Hz, 1H), 7.34-7.36 (m, 3H), 7.48 (d, *J* = 7.3 Hz, 1H), 8.19 (d, *J* = 8.8 Hz, 1H).

1-(4-(Benzo*[b]*thiophen-5-yl)phenyl)propan-1-ol, 12a.

Synthesised according to Method E using **12b** (0.35 g, 1.47 mmol) and a 1.0 M ethylmagnesium bromide solution in THF (1.91 mL, 1.91 mmol); yield: 0.34 g (85 %); R_f = 0.30 (PE / EtOAc, 2:1); the crude product was directly used in the next step without further purification and analysis.

1-(4-(*1H*-Indol-5-yl)phenyl)propan-1-ol, 13a.

Synthesised according to Method E using **13b** (0.50 g, 2.26 mmol) and a 1.0 M ethylmagnesium bromide solution in THF (2.94 mL, 2.94 mmol); yield: 0.51 g (89 %); R_f = 0.30 (PE / EtOAc, 2:1); the crude product was directly used in the next step without further purification and analysis.

3-(4-(6-(*tert*-Butyldimethylsilyloxy)naphthalen-2-yl)phenyl)pentan-3-ol, 21b.

Synthesised according to Method E using **21c** (6.07 g, 16.7 mmol) and EtMgBr (1 M, 18.4 mL, 18.4 mmol, 1.1 eq); yield: 1.37 g (21%); yellow solid; *Rf* = 0.73 (hexane / EtOAc, 7 : 3); the crude product was directly used in the next step without further purification and analysis.

(6-Bromonaphthalen-2-yloxy)(*tert*-butyl)dimethylsilane, 21d.

To a solution of 6-bromonaphthalen-2-ol (10 g, 44.8 mmol) and imidazole (3.4 g, 49.3 mmol, 1.1 eq) in dichloromethane was slowly added a solution of tert-butyldimethylsilyl chloride (7.4 g, 49.3 mmol, 1.1 eq) in dichloromethane. After being stirred for 4 h at rt the reaction mixture was poured into

water, extracted with dichloromethane, washed with water and brine, and dried over Na_2SO_4. Solvent removal under reduced pressure lead to a pale oil, which was purified by chromatography on silica gel; yield: 14.5 g, (96%); yellow oil; *Rf* = 0.63 (hexane); δ_H ($CDCl_3$, 500 MHz) 0.25 (s, 6H), 1.02 (s, 9H), 7.09 (dd, *J* = 2.2, *J* = 8.8 Hz, 1H), 7.15 (d, *J* = 2.2 Hz, 1H), 7.48 (dd, *J* = 2.2, *J* = 8.8 Hz, 1H), 7.56 (d, *J* = 8.8 Hz, 1H), 7.63 (d, *J* = 8.8 Hz, 1H), 7.92 (d, *J* = 2.2 Hz, 1H); δ_C ($CDCl_3$, 125 MHz) -4.3 (CH_3), 18.2 (C_q), 25.7 (CH_3), 114.9 (CH), 117.3 (C_{Br}), 123.1 (CH), 128.3 (CH), 128.4 (C_q), 129.4 (CH), 129.6 (CH), 130.3 (CH), 133.1 (C_q), 153.8 (C_{OR}); MS (ESI): m/z = 337 [M^+ + H].

6-(4-(3-(*1H*-Imidazol-1-yl)pentan-3-yl)phenyl)naphthalen-2-ol, 21.

To a solution of the silyl-protected phenol **21a** (crude product, 2.5 mmol) in anhydrous tetrahydrofuran (25 mL) was added tetrabutylammonium fluoride (3 mL, 1 M) and the solution was stirred for 4 h. The reaction was terminated with the addition of methanol and the solvent was removed under reduced pressure. Then, the desired product was purified by chromatography on silica gel; yield = 50 mg (6%); brown solid; R_f = 0.31 (EtOAc/MeOH 95/5); IR (ATR) υ (cm^{-1}) 2975 (w), 2874 (w), 1602 (s), 15 10 (m), 1498 (m), 1251 (s), 1205 (s), 858 (s), 831 (s); δ_H ($CDCl_3$, 500 MHz) 0.65 (t, *J* = 7.3 Hz, 6H), 2.27-2.31 (m, 4H), 6.94 (bs, 1H), 7.06 (bs, 1H), 7.10 (dd, *J* = 2.4 Hz, *J* = 8.8 Hz, 1H), 7.14 (d, *J* = 2.4 Hz, 1H), 7.22 (d, *J* = 8.8 Hz, 2H), 7.70-7.76 (m, 4H), 7.80 (bs, 1H), 7.82 (d, *J* = 8.8 Hz, 1H), 8.07 (bs, 1H), 9.84 (s, 1H); δ_C ($CDCl_3$, 125 MHz) 7.6 (CH_3), 28.9 (CH_2), 65.7 (CH), 108.4 (CH), 117.1 (CH), 118.4 (CH), 119.0 (CH), 125.0 (CH), 125.0 (CH), 126.2 (CH), 126.6 (CH), 126.7 (CH), 127.9 (C_q), 128.1 (C_q), 129.7 (CH), 133.5 (C_q), 133.8 (C_q), 139.0 (C_q), 142.9 (CH), 155.5 (C_{OH}); MS (ESI): *m/z* =357 [M^++H].

Biological Assays. CYP17 Preparation and Assay. As source of human CYP17, our *E. coli* system[17] (co-expressing human CYP17 and NADPH-P450 reductase) was used and the assay was performed as previously described[7] taking unlabeled progesterone as substrate and applying HPLC with UV-detection for separation.

Inhibition of Hepatic CYP Enzymes. The recombinantly expressed enzymes from baculovirus-infected insect microsomes (BD Supersomes™) were used and the manufacturer's instructions (www.gentest.com) were followed.

Inhibition of CYP11B1. V79MZh11B1 cells expressing the respective human enzyme were used and our assay procedure using [4-^{14}C]-11-deoxycorticosterone was applied.[19]

Molecular Modelling. All molecular modelling studies were performed on Intel® P4 CPU 3.00 GHz running Linux Suse 10.1.

Ligands. The structures of the inhibitors were built with SYBYL 7.3.2 (Sybyl, Tripos Inc., St. Louis, Missouri, USA) and energy-minimised in MMFF94s force-field[24] as implemented in Sybyl. The resulting geometries for our compounds were then subjected to ab initio calculation employing the B3LYP functional[25] in combination with a 6-31G* basis set using the package Gaussian03 (Gaussian, Inc., Pittsburgh, PA, USA, 2003).

Docking. Various inhibitors of Tables 1 and 2 were docked into our CYP17 homology model by means of the GOLD v 3.0.1 software.[20] Since the GOLD docking program allows flexible docking of the compounds, no conformational search was employed to the ligand structures. GOLD gave the best poses by a genetic algorithm (GA) search strategy, and then various molecular features were encoded as a chromosome.

Ligands were docked in 50 independent GA runs using GOLD. Heme iron was chosen as active site origin, while its radius was set equal to 19 Å. The automatic active site detection was switched on. Furthermore, a distance constraint of a minimum of 1.9 and a maximum of 2.5 Å between the sp^2-hybridised nitrogen of the imidazole and the iron of the heme was set. Additionally, some of the GOLDSCORE parameters were modified to improve the weight of hydrophobic interaction and of the coordination between iron and nitrogen. The genetic algorithm default parameters were set as suggested by the GOLD authors.[20] On the other hand, the annealing parameters of fitness function were set at 3.5 Å for hydrogen bonding and 6.5 Å for van-der-Waals interactions.

All 50 poses for each compound were clustered with ACIAP[22] and the representative structure of each significant cluster was selected. The quality of the docked representative poses was evaluated based on the GOLDSCORE values, which give a good measure of the found binding mode, and on visual inspection of the putative binding modes of the ligands, as outcome of docking simulations and cluster analysis.

Acknowledgment.

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3.3 Steroidogenic cytochrome P450 (CYP) enzymes as drug targets: Combining substructures of known CYP inhibitors leads to compounds with different inhibitory profile

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Paper III

Abstract: Four out of six CYP enzymes involved in steroid biosynthesis are very interesting targets for the development of new drugs in order to treat a variety of severe illnesses. Herein we report on a novel approach for the discovery of hit compounds using new combinations of substructures of known CYP inhibitors. The synthesis of new scaffolds and their biological evaluation regarding inhibition of CYP17, CYP19, CYP11B1 and CYP11B2 is described. Thus, the very active (IC_{50} = 114 and 100 nM) and selective (IC_{50} > 1000 nM) CYP11B2 inhibitors, compounds **4** and **5**, were obtained as well as the dual inhibitor **3,** reducing the activities of CYP19 and CYP11B2.

Introduction

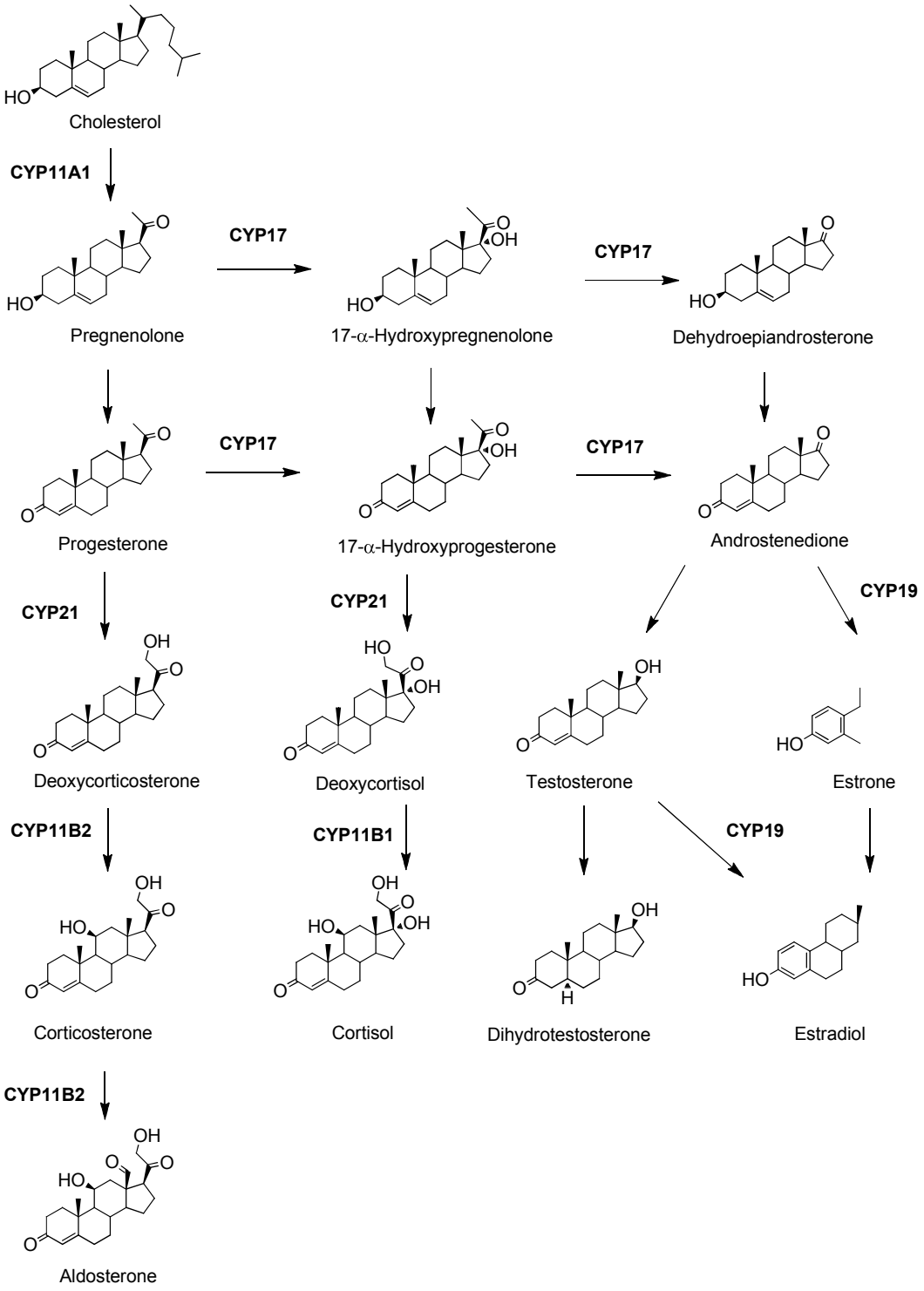
Cytochrome P450s are a large family of heme-containing enzymes, catalysing a variety of reactions including hydroxylations, epoxidations, *N*-dealkylations, *O*-dealkylations and *S*-oxidation. Although exceptions can be found, most of them act as monooxygenases, catalysing the insertion of a single oxygen atom into their substrate. Regarding their function, they can be divided into two groups: the hepatic enzymes unspecifically convert endogenous and exogenous compounds, like drugs, into more hydrophilic products. The other group consists of enzymes with a very specific role in the biosynthesis of bioactive molecules like hormones. In this work we will focus our attention on the latter group and therein especially on the CYP enzymes involved in the human steroid biosynthesis. These enzymes show in contrast to the hepatic CYPs high substrate specificity and are therefore principally suitable as drug targets.

In steroidogenesis six CYP enzymes are involved (Scheme 1), namely CYP11A1 (cholesterol side chain cleavage enzyme, SCC, desmolase), CYP21 (steroid-21-hydroxylase), CYP17 (17α-hydroxylase-C17,20-lyase), CYP19 (aromatase), CYP11B1 (steroid-11beta-hydroxylase) and CYP11B2 (aldosterone-synthase). As inhibition of CYP11A1 affects formation of all steroid hormones it is not appropriate as drug target. The same is true for CYP21 being involved in the biosynthesis of both gluco- and mineralocorticoids.

So far, the most successful example of targeting CYP enzymes is presumably the introduction of aromatase (CYP19) inhibitors for the treatment of hormone dependent breast cancer (HDBC). With these drugs it was possible to overcome the drawbacks of the anti-estrogen tamoxifen, which used to be the standard treatment of HDBC for years, although having many side effects due to its affinity to the estrogen receptor. In 1977 the aromatase inhibitor formestane was proven to be an efficient therapeutic for HDBC [1]. In the following years some steroidal and non-steroidal inhibitors have been approved. Although there are several inhibitors available today it is worthwhile to engage in the development of new entities in this field for several reasons, e.g. new estrogen dependent indications like endometriosis are emerging.

A similar approach was followed for the treatment of hormone dependent prostate cancer (HDPC). After *Huggins* and *Hodges* had shown in 1941 that androgen deprivation is effective against a high percentage of prostate tumours [2,3], the surgical castration became the standard treatment. Later "medical castration" using GnRH analogues (agonists [4] and very recently also antagonists [5]) came up as an alternative. However, neither treatment is able to affect adrenal androgen production. Testicular and adrenal androgen production could be suppressed by CYP17 inhibitors. The first clinically administered, rather unselective CYP17 inhibitor was the antimycotic ketoconazole. It turned out to be highly effective against prostate tumours [6,7], but finally was not accepted as it showed severe side effects such as liver toxicity [8]. The basic problem that CYP17 inhibitors also reduce glucocorticoid levels (Scheme 1) could be solved in these clinical studies with ketoconazole.

Low dose treatment regimens are capable of reducing testosterone levels but do not affect glucocorticoid concentrations [9].Very recently, the steroidal CYP17 inhibitor abiraterone has passed phase II clinical trials. It showed high activity and seems to have no dose-limiting toxicity [10]. Nevertheless, steroidal drugs are known for their potential side effects due to their affinities for other steroid hormone receptors. Therefore we investigated extensively in the development of non-steroidal CYP17 inhibitors.

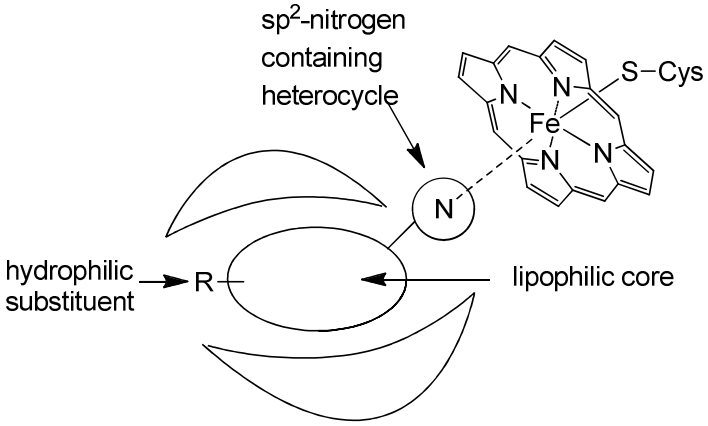


Scheme 1. CYP enzymes in steroid biosynthesis

The third enzyme to be regarded as a potential drug target is aldosterone synthase (CYP11B2). As the aldosterone antagonists spironolactone and eplerenone have been proven to reduce mortality in patients suffering from congestive heart failure and myocardial fibrosis [11,12], we expect the inhibition of aldosterone formation to be a milestone for the treatment of these diseases. Although no selective inhibitor is on the market yet, a few compounds, like fadrozole or ketoconazole, are known to deprive aldosterone production [13,14]. However, these compounds unselectively interact with other CYP enzymes and show only moderate activity. Very recently, we reported on the discovery and development of highly active and selective CYP11B2 inhibitors with oral bioavailability in the rat [15].

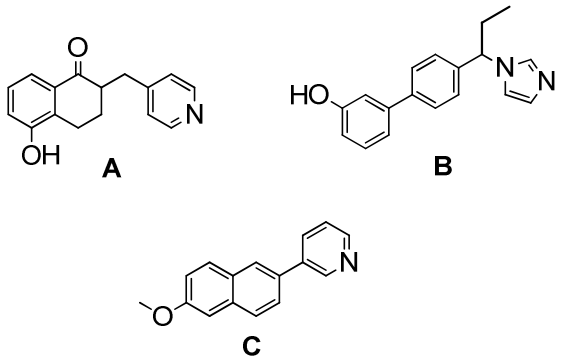
As fourth steroidogenic CYP enzyme 11-β-steroidhydroxylase (CYP11B1) has recently been propagated as a novel target for diseases with abnormal cortisol levels like *Cushing´*s syndrome [14], and first inhibitors have been described [16-18].

In the last two decades we have discovered a large number of highly active and selective CYP19 [19-22], CYP17 [23-26] and CYP11B2 [15,16,17,27,28] inhibitors, respectively. All of them contain a heterocycle with sp^2-hybridized nitrogen, which is complexing the heme iron of the active site of the enzyme (Scheme 2).



Scheme 2. Proposed model for CYP inhibitors: Active site of the enzyme (heme and substrate binding site)

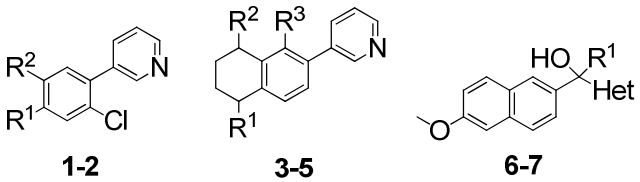
This heterocycle is substituting directly or via a linker a core structure consisting of at least two more rings. Furnished with appropriate substituents, highly active inhibitors (**A**, **B** and **C**) of the corresponding enzymes were obtained (Scheme 3).



Scheme 3. Highly active inhibitors of CYP19 (**A**)[21], CYP17 (**B**)[26], and CYP11B2 (**C**)[27]

In the following we describe a novel approach for the discovery of new hit compounds. Using our CYP19 (**A**), CYP17 (**B**) and CYP11B2 (**C**) inhibitors, we here have studied new combinations of their substructures (core: naphthalene, tetrahydronaphthalene, biphenyl; linker: no, methylene, substituted methylene; heterocycle: 3-pyridine, 4-pyridine, 1-imidazole) leading to compounds **1** – **7** (Table 1). The synthesis and biological evaluation regarding their activity and selectivity toward the four CYP enzymes is reported.

Table 1. List of synthesized compounds **1**-**7**.



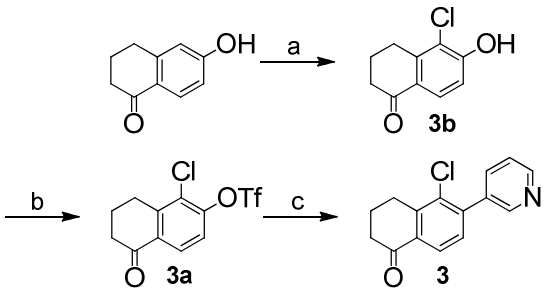
Compound	R^1	R^2	R^3	Het
1	2-OMe-Ph	H		
2	H	3-OMe-Ph		
3	=O	H	Cl	
4	H	=O	H	
5	H	OH	H	
6	Et			3-Py
7	H			4-Py

Chemistry

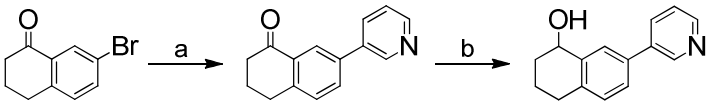
The syntheses of compounds **1** – **7** are shown in schemes 4 - 7. In our aim to explore different core structures with improved activity and selectivity toward the most important steroidogenic CYP enzymes, a variety of structural quite different compounds was synthesized. They can be divided in biphenyls, naphthalenes and tetralones linked either directly or via a methylene bridge to heme-complexing pyridine.

The synthesis of biphenyls C-C bonds was carried out by palladium-catalyzed *Suzuki* coupling reactions using commercially available boronic acids and aryl bromides (**4**, **1b**, **2b**) or triflates (**3**, **1**, **2**), which were synthesized from the corresponding alcohol (**3a**, **1a**, **2a**).

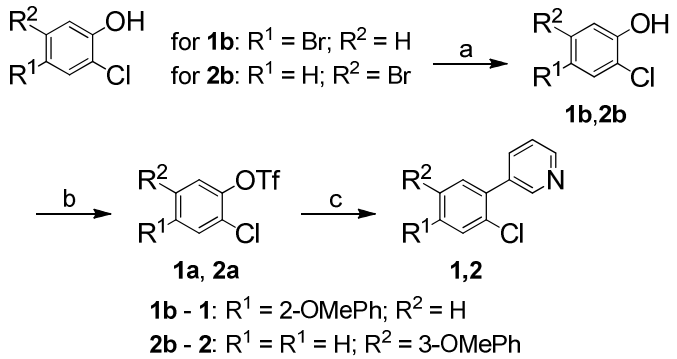
Following the procedure of *Weinstock* [29 28] and *Bravo* [30 29], a chlorine substituent was introduced in compound **3b**. Reduction of the tetralone **4** led to its corresponding alcohol **5**. The introduction of a methylene bridge between the naphthalene moiety and the pyridyl group (compounds **6** and **7**) was obtained by a bromine/lithium-exchange in 2-bromo-6-methoxynaphtalene and nucleophilic addition of the resulting organolithium compound to either nicotinealdehyde to afford the secondary alcohol **6** or to **6a** yielding the tertiary alcohol **7**. Compound **6a** was prepared by conversion of nicotinoyl chloride to its *Weinreb* amide **6b** and the subsequent introduction of an ethyl group by *Grignard* reaction with EtMgBr.



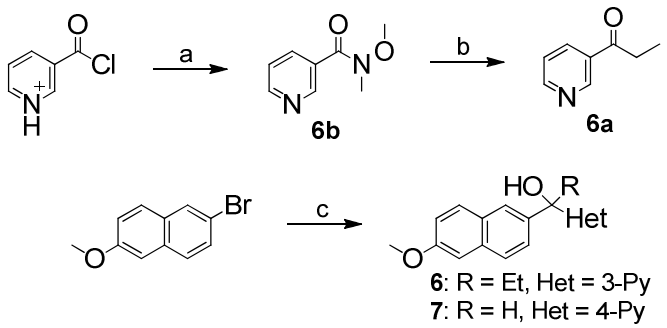
Scheme 4. Synthesis of 6-substituted tetralone derivative
Reagents and conditions: a) *tert*-BuOCl, DCM, 0 °C, 2h; b) Method A: Tf_2O, pyridine, DCM, 0 °C – rt, 3h; c) Method B: 3-pyridinylboronic acid, $Pd(PPh_3)_4$, Na_2CO_3, toluene, H_2O, reflux, 8 h.



Scheme 5. Synthesis of 7-substituted tetralone derivatives. Reagents and conditions: a) Method B: 3-pyridinylboronic acid, $Pd(PPh_3)_4$, Na_2CO_3, toluene, H_2O, reflux, 8 h; b) $NaBH_4$, MeOH, THF, 0 °C – rt, 2h.



Scheme 6. Synthesis of biphenylic derivatives. Reagents and conditions: a) Method B: $R^1B(OH)_2$ or $R^2B(OH)_2$, $Pd(PPh_3)_4$, Na_2CO_3, toluene, H_2O, reflux, 8 h; b) Method A: pyridine, Tf_2O, DCM, 0 °C – rt, 3h; c) Method B: 3-pyridinylboronic acid, $Pd(PPh_3)_4$, Na_2CO_3, toluene, H_2O, reflux, 8 h.



Scheme 7. Synthesis of bridged naphthalene derivatives. Reagents and conditions: a: NEt_3, $NH(OMe)_2 \times HCl$, DCM, rt, 16 h; b: EtMgBr, THF, rt, 8h; c: Method C:, *tert*-BuLi, THF, -78 °C, then **6a** or isonicotinaldehyde, -78 °C – 0 °C, 2h.

Biological Results

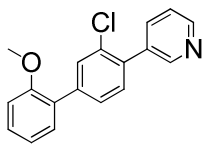
Inhibition of human CYP17 was determined by performing our previously described assay [30]. As source of human CYP17, *E. coli* coexpressing human CYP17 and NADPH-P450 reductase was used [31]. After homogenization, the 50.000 g sediment was incubated with progesterone (25 µM) and inhibitor. Separation of the product was performed by HPLC using UV-detection. The IC_{50} values determined for compounds **1**-**7** are shown in Table 2 with well known reference compounds.
All of the seven compounds showed only very little inhibitory activity toward CYP17 with IC_{50} values > 1000 nM.

For the determination of the CYP11B2 inhibitory activities, V79MZh11B2 cells expressing human CYP11B2 were used as well as [^{14}C]-labeled 11-deoxycorticosterone (300 nM) as substrate. The product formation was monitored by HPTLC using a phosphoimager [3,16,32-35]. The IC_{50} values determined for compounds **1 - 7** are shown in Table 2. While compounds **1, 2** and **6** showed only very little inhibition (IC_{50} values > 1000 nM) of the mineralocorticoid-forming enzyme, a high inhibitory activity could be observed for compounds **3** – **5** and **7** (IC_{50} = 10 – 140 nM). Compound **3** turned out to be the most active one showing an IC_{50} of 10 nM.

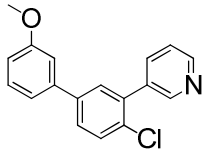
Determination of CYP11B1 inhibition has been performed in analogy to the procedure for CYP11B2 described above with V79MZh11B1 cells. The IC_{50} values determined for compounds **1 - 7** are shown in Table 2. Compounds **1**, **2**, **4** and **5** showed only very little (IC_{50} > 1000 nM) and compounds **3**, **6** and **7** rather low (IC_{50} = 391 – 495 nM) activity toward CYP11B1.

The *in vitro* aromatase inhibitory activity was evaluated using the microsomal fraction of human placental tissue [36] according to a described procedure [37,38]. Labeled [1β-^{3}H]androstenedione (500 nM), NADPH-generating system and inhibitor were incubated. The formed ^3H_2O was measured [39]. The IC_{50} values determined for compounds **1**-**7** are shown in Table 2. Here compound **3** turned out to be a strong aromatase inhibitor (IC_{50} = 19 nM). Compounds **1**, **2** and **4** – **7** showed only very little activity (IC_{50} > 1000 nM).

Table 2. Inhibition of CYP19, CYP17, CYP11B2 and CYP11B1 by compounds **1**-**7**.



1



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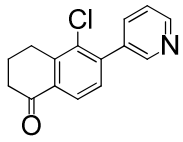
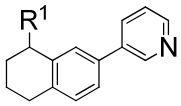
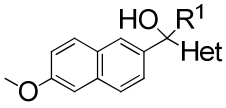


3



4-5



6-7

Compound	Structure		IC_{50} [nM]			
	R^1	Het	CYP19 [a,d]	CYP17 [a,b]	CYP11B2 [a,c]	CYP11B1 [a,c]
1			> 1000	> 5000	> 1000	> 5000
2			> 1000	> 1000	> 1000	> 1000
3			19	> 1000	10	495
4	=O		> 5000	> 5000	114	1262
5	OH		> 1000	> 50,000	100	2152
6	Et	3-Py	> 1000	> 50,000	1144	429
7	H	4-Py	> 1000	> 1000	140	391
KTZ [e]				2780	67	127
FDZ [e]					1	10
ABT [e]				72	1751	1608

[a] Data shown were obtained by performing the tests in duplicate. The deviations were within < ±5 %.
[b] Concentration of progesterone (substrate) was 25 µM
[c] Concentration of 11-deoxycorticosterone (substrate) was 100 nM
[d] Concentration of androstenedione (substrate) was 500 nM
[e] **KTZ**: ketoconazole; **FDZ**: fadrozole; **ABT**: abiraterone

Discussion

Regarding the main core, the compounds can be divided in biphenylic structures (**1** and **2**), in tetrahydronaphthalenes (**3** – **5**) and in naphthalenes (**6** and **7**). In compounds **1** – **5** the heterocycle is bound directly to the core, while in compound **6** and **7** a hydroxy substituted methylene bridge, carrying an additional ethyl group in compound **7**, is introduced,

It becomes apparent, that the biphenyls, which are derived from the highly active CYP17 inhibitor **B**, only show very little inhibition of all target enzymes (IC_{50} > 1000 nM) independent from their substitution pattern. From this it can be concluded that for the biphenyls, the kind and orientation of the heterocycle is very important, as well as the methylene spacer between the core and the heterocycle.

In the class of the naphthalenes we found a very active compound (**7**). Derived from compound **C** - a strong CYP11B2 inhibitor - the modified structures **6** and **7** showed very interesting effects: By introducing a hydroxy substituted methylene spacer connecting the core with a 4-pyridine, compound **7** keeps its inhibitory activity for CYP11B2 (IC_{50} = 140 nM) with a moderate selectivity to CYP11B1 (IC_{50} = 429 nM) and high selectivity toward CYP17 and CYP19 (IC_{50} > 1000 nM). On the contrary,

the inhibitory activity of the naphthalenes is decreased by introduction of an additional ethyl substituent at the methylene bridge and moving the 4-pyridyl nitrogen into the 3-position as shown by compound **6**. This compound exhibits moderate activity toward CYP11B1 (IC_{50} = 491 nM) and very low activity toward the other enzymes (IC_{50} > 1000 nM).

The tetralone **3** derived from aromatase inhibitor **A**, which is connected to the pyridine moiety in a different way compared to the parent compound, shows a very interesting activity profile: As expected, this compound is highly active regarding its inhibition of aromatase (IC_{50} = 19 nM), but, additionally, it is the most potent CYP11B2 inhibitor of this series (IC_{50} = 10 nM). It is very selective toward CYP17 (IC_{50} > 1000) and shows only low inhibition of CYP11B1 (IC_{50} = 495 nM). Therefore this compound might be an attractive starting point for the development of dual inhibitors.

In contrast to this finding, the tetralone **4** and its respective alcohol **5** completely loose their aromatase inhibitory activity (IC_{50} > 1000 nM), while keeping their potency toward CYP11B2 (IC_{50} = 114 resp. 100 nM). Furthermore only very little activity toward CYP11B1 and CYP17 could be observed. Therefore these two compounds can be regarded as highly active and selective CYP11B2 inhibitors.

Conclusion and outlook

From the data shown in this paper it becomes apparent that our new approach for the discovery of novel hit compounds targeting steroidogenic CYP enzymes was successful. Derived from the aromatase (CYP19) inhibitor **A**, we succeeded in finding a new lead structure for the development of novel inhibitors of CYP11B2, namely the tetralone derivatives **4** and **5**. Furthermore a dual inhibitor reducing the activities of CYP19 and CYP11B2 (compound **3**) could be identified. The optimization of these new compounds is presently under investigation.

Experimental Section

CYP17 preparation and assay

As source of human CYP17, our *E. coli* system [40] coexpressing human CYP17 and NADPH-P450 reductase was used. The assay was performed as previously described [30] using unlabeled progesterone as substrate and applying HPLC with UV-detection for separation.

CYP19 preparation and assay

As source of human CYP19, microsomal preparations of human placenta were used [36,41]. The assay was performed using the $^{3}H_2O$-method as described [37,38] using [1β-^{3}H]androstenedione and unlabelled androstenedione as substrate and monitored by β-counter detection.

Inhibition of CYP11B1 and CYP11B2

V79MZh11B1 or V79MZh11B2 cells expressing CYP11B1 or CYP11B2 [33-35] were used and our assay procedure with [4-^{14}C]-11-deoxycorticosterone was applied [16,32] using [^{14}C]-labeled 11-deoxycorticosterone as substrate and monitored by TLC and by use of a phosphoimaging.

Chemistry Section

General Experimental

Melting points were determined on a Mettler FP1 melting point apparatus and are uncorrected. IR spectra were recorded neat on a Bruker Vector 33FT-infrared spectrometer. ^{1}H-NMR spectra were measured on a Bruker DRX-500 (500 MHz). Chemical shifts are given in parts per million (ppm), and TMS was used as an internal standard for spectra obtained in $CDCl_3$. All coupling constants (*J*) are given in Hz. ESI (electrospray ionization) mass spectra were determined on a TSQ quantum (Thermo Electron Corporation) instrument. Elemental analyses were performed at the Department of Instrumental Analysis and Bioanalysis, Saarland University. Column chromatography was performed using silica gel 60 (50-200 µm), and reaction progress was monitored by TLC analysis on Alugram® SIL G/UV_{254} (Macherey-Nagel). Boronic acids and bromoaryls used as starting materials were obtained commercially (CombiBlocks, Chempur, Aldrich, Acros) and used without further purification.

Method A: Synthesis of Triflates.

To a solution of the corresponding alcohol and pyridine in dry DCM (10 mL / mmol), trifluoromethanesulfonic anhydride was carefully added over 1 min. at 0°C. The reaction mixture was stirred at room temperature for 3 h. Afterwards, excess trifluoromethanesulfonic anhydride was neutralized with Na_2CO_3, and the crude was washed two times with water. The organic phase was dried over Na_2SO_4 and concentrated. Purification of the residue was performed by flash chromatography using SiO_2.

3-Chloro-2'-methoxy-biphenyl-4-yl trifluoromethanesulfonate (1a)

Synthesized from **1b** (0.85 g, 3.6 mmol), pyridine (0.30 mL, 3.7 mmol) and trifluoromethanesulfonic anhydride (1.22 mL, 7.24 mmol) in dry DCM (10 mL) according to Method A. Yield: 85 %. The obtained crude triflate was used directly for the *Suzuki* coupling without further purification.

4-Chloro-3'-methoxy-biphenyl-3-yl trifluoromethanesulfonate (2a)

Synthesized from **2b** (0.93 g, 4.0 mmol), pyridine (0.32 mL, 4.0 mmol) and trifluoromethanesulfonic anhydride (1.35 mL, 8.02 mmol) in dry DCM (10 mL) according to Method A. Yield: 82 %. The obtained crude triflate was used directly for the *Suzuki* coupling without further purification.

1-Chloro-5-oxo-5,6,7,8-tetrahydronaphthalen-2-yl trifluoromethanesulfonate (3a)

Synthesized from **3b** (6.50 g, 31.2 mmol), pyridine (3.76 mL, 46.7 mmol) and trifluoromethanesulfonic anhydride (10 mL, 59.0 mmol) in dry DCM (20 mL) according to Method A. Yield: 33 %. ^{1}H NMR ($CDCl_3$, 500 MHz): δ_H (ppm) = 2.15 (q, *J* = 6.4 Hz, 2H), 2.61 (t, *J* = 6.5 Hz, 2H), 3.02 (t, *J* = 6.2 Hz, 2H), 7.27 (d, *J* = 8.7 Hz, 1H), 8.00 (d, *J* = 8.7 Hz, 1H). ^{13}C NMR ($CDCl_3$, 125 MHz): δ_C (ppm) = 21.98, 27.43, 37.89, 114.4, 117.3, 119.8, 120.7, 127.2, 127.3, 127.9, 133.4, 144.8, 148.9, 195.8.

Method B: Suzuki-Coupling.

The corresponding naphthalene or benzene derivative and the boronic acid were dissolved in toluene (20 mL) and aq. Na_2CO_3 (2.0 M, 5.0 mL). The mixture was deoxygenated under reduced pressure and flushed with N_2. After having repeated this cycle three times, $Pd(PPh_3)_4$ (5 mol%) was added, and the resulting suspension was heated under reflux for 8 h. After cooling, the phases were separated and the water phase was extracted two times with EtOAc. The combined organic extracts were dried over Na_2SO_4, and concentrated under reduced pressure. The purification was performed by flash chromatography using SiO_2.

3-(3-Chloro-2'-methoxy-biphenyl-4-yl)-pyridine (1)

Synthesized from **1a** (0.98 g, 2.7 mmol) and 2-pyridinylboronic acid (0.49 g, 4.0 mmol) according to Method B. Pale oil. Yield: 72 %. IR (ATR) υ (cm^{-1}) = 2927, 1601, 1466, 1435, 1412,1251, 1181, 1082, 1026, 833, 807, 752, 714, 680; ^{1}H NMR ($CDCl_3$, 500 MHz): δ_H (ppm) = 3.87 (s, 3H), 7.01-7.08 (m, 2H), 7.34-7.40 (m, 4H), 7.53 (d, *J* = 7.8 Hz, 1H), 7.70 (s, 1H), 7.86 (d, *J* = 7.8 Hz, 1H), 8.64 (d, *J* = 3.8 Hz, 1H), 8.75 (s, 1H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ_C (ppm) = 55.60, 111.3, 120.9, 124.3, 124.6, 122.9, 128.3, 128.4, 128.5, 128.6, 129.4, 130.7, 130.8, 131.3, 136.9, 140.1, 148.6, 150.1, 156.4; MS (ESI): m/z = 296.2 $[M+H]^+$.

3-Chloro-2'-methoxy-biphenyl-4-ol (1b)

Synthesized from 5-bromo-2-chlorophenol (1.00 g, 4.82 mmol) and 3-methoxyphenylboronic acid (1.10 g, 7.23 mmol) according to Method B. Yield: 75 %. ^{1}H NMR ($CDCl_3$, 500 MHz): δ_H (ppm) = 3.74 (s, 3H), 5.49 (s, 1H), 6.89 (d, *J* = 8.2 Hz, 1H), 6.93 (t, *J* = 7.4 Hz, 1H), 6.97 (d, *J* = 8.2 Hz, 1H), 7.19 (dd, *J* = 1.6 Hz, *J* = 7.4 Hz, 1H), 7.21-7.25 (m, 1H), 7.26 (dd, *J* = 2.1 Hz, *J* = 8.4 Hz, 1H), 7.43 (d, *J* = 2.1 Hz, 1H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ_C (ppm) = 55.53, 111.2, 115.7, 119.1, 120.8, 128.7, 128.9, 129.6, 129.8, 130.5, 131.9, 150.3, 156.3.

3-(4-Chloro-3'-methoxy-biphenyl-3-yl)-pyridine (2)

Synthesized from **2a** (0.58 g, 2.3 mmol) and 3-pyridinylboronic acid (0.43 g, 3.5 mmol) according to Method B. Pale oil. Yield: 83 %. IR (ATR) υ (cm^{-1}) = 2924, 1583, 1465, 1410, 1319, 1288, 1090, 1043, 1011, 874, 780, 713, 696; ^{1}H NMR ($CDCl_3$, 500 MHz): δ_H (ppm) = 3.86 (s, 3H), 6.93 (dd, *J* = 2.3 Hz, *J* = 8.2 Hz, 1H), 7.11 (s, 1H), 7.17 (d, *J* = 7.7 Hz, 1H), 7.35-7.41 (m, 2H), 7.53-7.56 (m, 3H), 7.84-7.86 (m, 1H), 8.66 (d, *J* = 3.8 Hz, 1H), 8.75 (s, 1H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ_C (ppm) = 55.59, 112.3, 120.9, 122.8, 124.7, 128.3, 128.6, 129.4, 130.7, 131.0, 132.3, 133.3, 136.9, 140.0, 141.2, 148.6, 150.1, 156.4; MS (ESI): m/z = 296.2 $[M+H]^+$.

4-Chloro-3'-methoxy-biphenyl-3-ol (2b)

Synthesized from 5-bromo-2-chlorophenol (1.00 g, 4.82 mmol) and 3-methoxyphenylboronic acid (1.10 g, 7.23 mmol) according to Method B. Yield: 82 %. ^{1}H NMR ($CDCl_3$, 500 MHz): δ_H (ppm) = 3.78 (s, 3H), 5.53 (s, 1H), 6.82 (dd, *J* = 2.4 Hz, *J* = 8.2 Hz, 1H), 6.99-7.02 (m, 2H), 7.05 (d, *J* = 7.7 Hz, 1H), 7.17 (d, *J* = 2.0 Hz, 1H), 7.25-7.29 (m, 2H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ_C (ppm) = 55.30, 112.7, 113.2, 114.8, 119.1, 119.5, 120.2, 129.1, 129.8, 141.3, 141.7, 151.4, 159.9.

5-Chloro-6-(pyridin-3-yl)-3,4-dihydronaphthalen-1(2H)-one (3)

Synthesized from **3a** and 3-pyridinylboronic acid according to Method B. Orange oil. Yield: 86 %. ^{1}H NMR ($CDCl_3$, 500 MHz): δ_H (ppm) = 2.15 (m, 2H), 2.62 (t, *J* = 7.2 Hz, 2H), 3.04 (t, *J* = 6.2 Hz, 2H), 7.24 (d, *J* = 7.9 Hz, 1H), 7.34 (dd, *J* = 4.8 Hz, *J* = 7.9 Hz, 1H), 7.99 (m, 2H), 8.60 (d, *J* = 4.8 Hz, 1H), 8.61 (s, 1H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ_C (ppm) = 22.18, 27.66, 38.10, 123.0, 125.7, 129.0, 132.7, 133.9, 135.2, 136.8, 142.0, 142.9, 149.1, 149.6, 197.1; MS (ESI): *m/z* = 258.0 $[M+H]^+$.

7-(Pyridin-3-yl)-3,4-dihydronaphthalen-1(2H)-one (4)

Synthesized from 7-Bromo-3,4-dihydro-2*H*-naphthalen-1-one and 3-pyridinylboronic acid according to Method B. Pale oil. Yield: 89 %. IR (ATR) υ (cm^{-1}) = 3028, 2940, 1677, 1608, 1427, 1325, 1252, 1220, 1180, 1028, 905, 808, 720, 555; ^{1}H NMR ($CDCl_3$, 500 MHz): δ_H (ppm) = 2.15-2.20 (m, 2H),

2.70 (dd, *J* = 6.0 Hz, *J* = 7.0 Hz, 2H), 3.02 (dd, *J* = 5.8 Hz, *J* = 6.1 Hz, 2H), 7.37-7.39 (m, 2H), 7.70 (dd, *J* = 2.1 Hz, *J* = 7.9 Hz, 1H), 7.91 (ddd, *J* = 1.5 Hz, *J* = 7.1 Hz, *J* = 7.9 Hz, 1H), 8.26 (d, *J* = 2.1 Hz, 1H), 8.60 (dd, *J* = 1.5 Hz, *J* = 4.9 Hz, 1H), 8.86 (d, *J* = 2.2 Hz, 1H); MS (ESI): m/z = 224.2 $[M+H]^+$.

7-(Pyridin-3-yl)-1,2,3,4-tetrahydronaphthalen-1-ol (5)

A suspension of sodium borohydride (0.090 g, 2.3 mmol) in MeOH (10 mL) was added to a solution of **4** (0.26 g, 1.16 mmol) in THF (10 mL) at 0 °C and stirred at room temperature over 2 h. The reaction mixture was extracted three times with EtOAc. The combined organic were extracts dried over Na_2SO_4 and concentrated. No further purification was necessary. Yellow oil. Yield: 96 %. IR (ATR) υ (cm^{-1}) = 3290, 2934, 1475, 1430, 1187, 1071, 1026, 988, 800, 710; ^{1}H NMR ($CDCl_3$, 500 MHz): δ_H (ppm) = 1.74-1.94 (m, 2H), 1.96-2.07 (m, 2H), 2.70-2.86 (m, 2H), 4.82 (dd, *J* = 4.7 Hz, *J* = 5.7 Hz, 1H), 7.15(d, *J* = 7.9 Hz, 1H), 7.27 (ddd, *J* = 1.0 Hz, *J* = 5.0 Hz, *J* = 8.2 Hz, 1H), 7.32 (dd, *J* = 1.9 Hz, *J* = 7.9 Hz, 1H), 7.66 (d, *J* = 1.9 Hz, 1H), 7.79 (ddd, *J* = 1.9 Hz, *J* = 2.5 Hz, *J* = 7.9 Hz, 1H), 8.45 (dd, *J* = 1.0 Hz, *J* = 4.7 Hz, 1H), 8.68 (dd, *J* = 1.0 Hz, *J* = 2.5 Hz, 1H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ_C (ppm) = 18.96, 28.94, 32.36, 67.79, 123.5, 125.8, 127.2, 129.6, 134.2, 135.3, 136.5, 137.2, 140.0, 147.7, 147.7; MS (ESI): *m/z* = 226.2 $[M+H]^+$.

5-Chloro-6-hydroxy-3,4-dihydronaphthalen-1(2H)-one (3b)

The synthesis of **3b** and the required *tert*-butyl hypochlorite was carried out according to Weinstock et al. [28] and Bravo et al. [29], respectively. Yield: 79 %. ^{1}H NMR ($CDCl_3$, 500 MHz): δ_H (ppm) = 2.03-2.12 (m, 2H), 2.55 (t, *J* = 6.4 Hz, 2H), 2.92 (t, *J* = 6.2 Hz, 2H), 6.23 (m, 1H), 7.97 (m, 1H).

N-Methoxy-N-methylnicotinamide (6b)

Nicotinoyl chloride hydrochloride (2 g, 11.2 mmol) and *N*-dimethoxy-methylamine HCl hydrochloride (12 g, 12.4 mmol) were dissolved in DCM (30 mL), and triethylamine (5.15 mL, 37.0 mmol) was added dropwise. After stirring at room temperature overnight, the reaction mixture was extracted with EtOAc and the solution concentrated under reduced pressure. Further purification was performed by flash chromatography using SiO_2. Yield: 82 %. ^{1}H NMR ($CDCl_3$, 500 MHz): δ_H (ppm) = 3.33 (s, 3H), 3.50 (s, 3H), 7.31 (ddd, *J* = 1.0 Hz, *J* = 4.9 Hz, *J* = 7.9 Hz, 1H), 7.97 (ddd, *J* = 2.0 Hz, *J* = 2.2 Hz, *J* = 7.9 Hz, 1H), 8.63 (dd, *J* = 1.6 Hz, *J* = 4.9 Hz, 1H), 8.90 (d, *J* = 1.6 Hz, 1H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ_C (ppm) = 33.05, 61.13, 122.8, 129.8, 136.0, 149.1, 151.2, 167.3.

1-(Pyridin-3-yl)propan-1-one (6a)

6b (1.3 g, 7.8 mmol) was dissolved in THF (20 mL), and ethylmagnesium bromide in THF (1.0 M, 10.1 mL, 10.1 mmol) was added dropwise. After stirring at room temperature for 8 h, water was added

and the mixture was stirred for additional 15 min. The layers were separated and the organic phase extracted three times with EtOAc. The combined organic extracts were dried over Na_2SO_4 and concentrated under reduced pressure. The purification was performed by flash chromatography using SiO_2. Yield: 62 %. ^{1}H NMR ($CDCl_3$, 500 MHz): δ_H (ppm) = 1.21 (t, *J* = 7.3 Hz, 3H), 2.99 (q, *J* = 7.3 Hz, 2H), 7.39 (ddd, *J* = 1.0 Hz, *J* = 4.8 Hz, *J* = 7.9 Hz, 1H), 8.21 (ddd, *J* = 1.9 Hz, *J* = 2.2 Hz, *J* = 7.9 Hz, 1H), 8.73 (dd, *J* = 1.0 Hz, *J* = 4.7 Hz, 1H), 9.14 (dd, *J* = 1.0 Hz, *J* = 2.2 Hz, 1H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ_C (ppm) = 7.78, 32.07, 123.6, 132.0, 135.2, 149.4, 153.2, 199.4.

Method C: Addition of the Heterocycle

2-Bromo-6-methoxynaphthalene (6 mmol) was dissolved in THF (20 mL) at -78° C and *tert*-BuLi in hexanes (1.5 M, 7.3 mL, 11 mmol) was added dropwise. This solution was added to a solution of ketone or aldehyde (5 mmol) in THF (20 mL) and the mixture was left stirring at 0° C for 2 h. Afterwards, water and EtOAc were added, the phases separated, and the aqueous phase was extracted three times with EtOAc. The combined organic extracts were dried over Na_2SO_4 and concentrated under reduced pressure. The purification was performed by flash chromatography using SiO_2.

1-(6-Methoxynaphthalen-2-yl)-1-(pyridin-3-yl)propan-1-ol (6)

Synthesized from **6a** according to Method C. Yellowish solid. Mp: 136.5°C. Yield: 43 %. IR (ATR) υ (cm^{-1}) = 2969, 1603, 1503, 1264, 1171, 1030, 985, 883, 804, 718, 560; ^{1}H NMR ($CDCl_3$, 500 MHz): δ_H (ppm) = 1.21 (t, *J* = 7.3 Hz, 3H), 2.99 (d, *J* = 7.3 Hz, 2H), 3.90 (s, 3H), 6.97-7.03 (m, 2H), 7.16-7.19 (m, 3H) 7.40-7.51 (m, 2H), 8.21 (ddd, *J* = 1.9 Hz, *J* = 2.2 Hz, *J* = 7.9 Hz, 1H), 8.73 (dd, *J* = 1.0 Hz, *J* = 4.7 Hz, 1H), 9.14 (dd, *J* = 1.0 Hz, *J* = 2.2 Hz, 1H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ_C (ppm) = 7.96, 34.17, 55.30, 105.5, 119.0, 123.1, 124.2, 125.2, 127.1, 128.4, 129.7, 133.6, 134.4, 141.0, 142.5, 147.5, 157.9; MS (ESI): *m/z* = 294.2 $[M+H]^+$.

(6-Methoxynaphthalen-2-yl)(pyridin-4-yl)methanol (7)

Synthesized from isonicotinaldehyde according to Method C. Yellowish solid. Mp: 167.8°C. Yield: 59 %. IR (ATR) υ (cm^{-1}) = 2926, 1603, 1507, 1270, 1169, 1056, 845, 772, 676; ^{1}H NMR ($CDCl_3$, 500 MHz): δ_H (ppm) = 3.69 (s, 3H), 5.65 (s, 1H), 6.90-6.93 (m, 2H), 7.16-7.19 (m, 3H), 7.47-7.51 (m, 2H), 7.55 (m, 2H), 8.28-8.30 (m, 2H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ_C (ppm) = 54.74, 73.82, 105.2, 118.5, 121.1, 124.0, 125.0, 126.7, 128.0, 128.9, 133.5, 138.3, 148.7, 153.5, 157.2; MS (ESI): *m/z* = 266.3 $[M+H]^+$.

Acknowledgement. UEH is grateful to the European Postgraduate School 532 (DFG) for a scholarship. We thank Profs Hermans (U. Maastricht) and Bernhardt (Saarland U.) for providing us with V79MZh11B1 and B2 cells, respectively, and Dr. Müller-Vieira for the CYP17 tests.

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3.4 Discovery of the first selective steroid-11β-hydroxylase (CYP11B1) inhibitors for the treatment of cortisol dependent diseases

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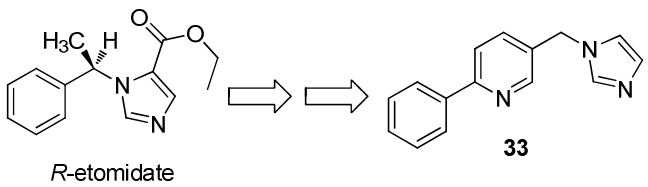
Paper IV

Abstract: Outgoing from an Etomidate based design concept, we succeeded in the development of a series of highly active and selective inhibitors of CYP11B1, the key enzyme of cortisol biosynthesis, as potential drugs for the treatment of Cushing´s Syndrome and related diseases. Thus compound **33** (IC_{50} = 152 nM) is the first CYP11B1 inhibitor showing selectivity toward the most important steroidogenic CYP enzymes aldosterone synthase (CYP11B2), the androgen forming CYP17 and aromatase (estrogen synthase, CYP19).



R-etomidate

Introduction

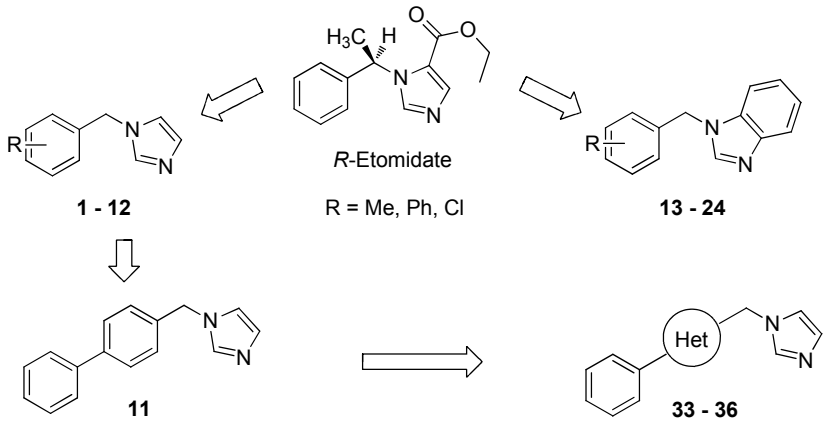
It is well-known that steroid hormones are essential for a large number of vitally important physiological processes. However, they are also associated with life-threatening diseases. Application of hormone receptor antagonists or biosynthesis inhibitors are regarded as therapeutic methods of choice. The biosynthetic pathways contain several established and potential drug targets. In the last decades, aromatase (CYP19) inhibitors were developed and continuously improved.[1-4] Nowadays, second and third generation inhibitors are used as first line therapeutics for hormone-dependent breast cancer.[5] Some 15 years ago, the first selective androgen synthase (CYP17) inhibitors were described,[6-8] and recently, their benefits for the treatment of castration refractory prostate cancer were demonstrated.[9] Research was not only focused on the formation of steroid hormones in their endocrine glands, but also on their activation in the target cell. Inhibition of steroid 5α-reductase is clinically well established for androgen-dependent diseases.[10-13] Experimental results with hydroxysteroid dehydrogenase (HSD) inhibitors are very encouraging for estrogen- and glucocorticoid-dependent diseases.[14-18]

Until some years ago, selective inhibitors of mineralo- and glucocorticoids were not in the focus of research efforts. This was due to the fact that the sequence identity between aldosterone synthase (CYP11B2) and cortisol synthase (steroid-11β-hydroxylase, CYP11B1) is very high (93 %),[19] and it was considered impossible to obtain selective inhibitors of one enzyme versus the other. Recently, however, we have been able to demonstrate that it is possible to selectively inhibit CYP11B2.[20-24] Further structural optimizations resulted in *in vivo* active compounds with selectivity factors reaching 1000 with regard to CYP11B1.[25,26] They could be candidates for the treatment of hyperaldosteronism, congestive heart failure, and myocardial fibrosis. Although there is a high medical need for drugs interfering with excessive glucocorticoid formation resulting in Cushing´s syndrome, there are only few inhibitors of CYP11B1 described so far.[27] Because of their unselective action, their application is associated with severe side effects: The CYP19 inhibitor aminoglutethimide, metyrapone, the antimycotics ketoconazole and fluconazole, and the hypnotic etomidate are also inhibitors of other adrenal and gonadal Cytochrom P450 (CYP) enzymes, and trilostane is a 3βHSD inhibitor.[28] In the present work, we report about the design, synthesis, and biological evaluation of the first selective (regarding CYP11B2, CYP17, and CYP19) inhibitors of human CYP11B1.

As the lead for the design of the compounds, the highly active CYP11B1 inhibitor *R*-etomidate was used (Scheme 1), in spite of the fact that it shows a stronger inhibition of CYP11B2. Indeed, this compound was one of the starting points recently used for the development of novel CYP11B2 inhibitors.[29] Therein, the authors have shown that modifications of the ester group by conformationally flexible substituents resulted in CYP11B2 selective compounds, while eliminating the ester led to moderate CYP11B1 selectivity. Therefore, we decided to either remove the ethyl-ester

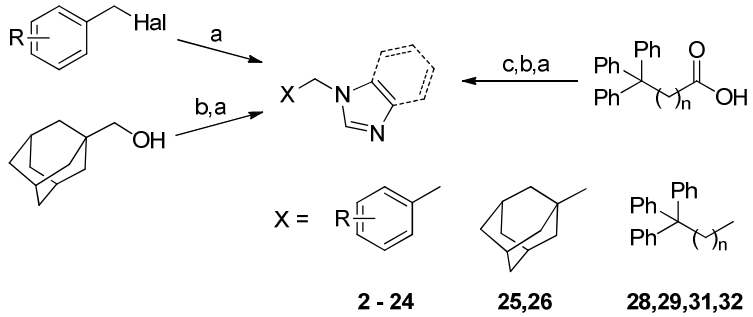
or to replace it by a rigid benzene nucleus. The chiral core was abolished by eliminating the methyl group as it has been shown that the alkyl group at the methylene bridge is a prerequisite for the hypnotic activity of etomidate derivatives.[30] As polar substituents at the phenyl moiety were shown to result in poor CYP11B1 inhibition,[29] methyl, chloro, and additional phenyl substituents were introduced (**2**–**24**). The most selective inhibitor obtained, **11**, was further optimized regarding selectivity versus CYP17 by exchange of one benzene ring by pyridine, furane, or thiophene (**33**–**36**).

Scheme 1. Inhibitor design concept.



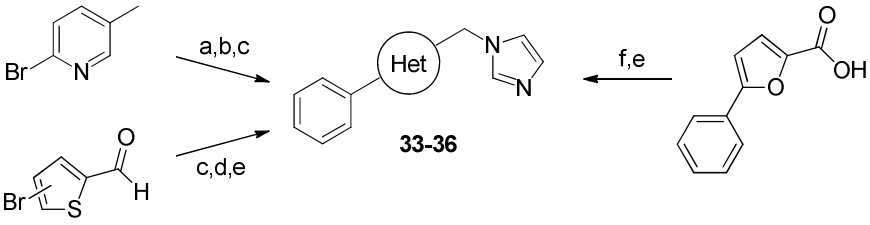
The syntheses of **2**–**36** are shown in Schemes 2 and 3. The starting point was commercially available *N*-benzylimidazole **1**. The preparation of the *N*-benzylimidazoles **2**–**12** and **33** and -benzimidazoles **13**–**24** was carried out via S_N reaction of the corresponding benzyl-halogenides. For the syntheses of **12**, **24,** and **33** palladium catalyzed Suzuki coupling and Wohl–Ziegler bromination were used. Preparation of the adamantanes **25** and **26** included tosylation of adamantan-1-yl-methanol followed by S_N reaction with imidazole or benzimidazole. S_N reaction of tritylchloride with imidazole or benzimidazole afforded **27** and **30**, carrying two additional phenyls at the methylene bridge (not shown). For the synthesis of **28, 29, 31,** and **32**, the corresponding carbonic acids were reduced to the alcohols with $LiAlH_4$ and the products further processed as described above. Compounds **34**–**36** were obtained by reduction of the aldehydes or carbonic acids to the primary alcohols with $NaBH_4$ or $LiAlH_4$ and subsequent CDI-assisted S_Nt reaction.

Scheme 2: Synthesis of compounds **2 - 26** and **28, 29, 31, 32**[a]



[a]Conditions: (a) **Method A**: imidazole or benzimidazole, K_2CO_3, DMF, 120°C, 2 h; (b) **Method E**: trifluoromethanesulfonic anhydride, pyridine, 0°C to rt, 3 h; (c) **Method C**: $LiAlH_4$, 0°C to rt, overnight.

Scheme 3: Synthesis of compounds **33 - 36**[a]



[a]Conditions: (a) NBS, DBPO, CCl_4, 90°C, 12h; (b) **Method A:** imidazole, K_2CO_3, acetonitrile, 90°C, 2 h; (c) **Method B:** phenylboronic acid, $Pd(PPh_3)_4$, Na_2CO_3, toluene/ MeOH/ H_2O, reflux, 5 h; (d) **Method D**: $NaBH_4$, MeOH, 2 h, rt; (e) **Method F**: CDI, acetonitrile, reflux, 8 h; (f) **Method C**: $LiAlH_4$, 0°C to rt, overnight.

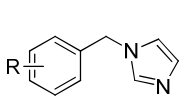
For the determination of CYP11B1 and CYP11B2 inhibition, V79MZ cells expressing either human CYP11B1 or CYP11B2 were used, and [^{3}H]-labelled 11-deoxycorticosterone as the substrate.[31,32] Metyrapone, etomidate, and ketoconazole served as references. The IC_{50} values determined for **1–36** are shown in Tables 1–3. All imidazoles **1–12**, **25**, **27–29,** and **33–36** strongly inhibited CYP11B1, mostly showing IC_{50} values below 100 nM, and compounds **8**, **25**, **27,** and **28** even reached values below 10 nM. Regarding the benzimidazoles, lower CYP11B1 inhibition than for the corresponding imidazoles was observed, some compounds showing IC_{50} values above 1000 nM. Most imidazoles exhibited inhibitory activity toward CYP11B2, while only some benzimidazoles (**14–18**, IC_{50} values: 107 nM–632 nM) showed marked inhibition of this enzyme.

Inhibition of CYP17 was investigated using a homogenate of *Escherichia coli* recombinantly expressing human CYP17 and progesterone as substrate.[8,33] At a concentration of 2000 nM, only **11** and **12** showed a marked inhibition of 40 and 52 %, while **25**, **35,** and **36** exhibited only weak effects with inhibition values around 20 %. All other substances showed no inhibition (data not shown). Inhibitory effects toward CYP19 were determined using human placental microsomes and [1β-^{3}H]androstenedione as substrate.[8] At a concentration of 500 nM, compounds **6, 9, 10, 27–29, 34,** and

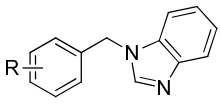
36 showed inhibition values above 38 %, while compounds **3**, **4, 8, 12, 25, 26,** and **35** exhibited little activity (12–28 %) and all other substances showed no inhibition (data not shown).

In the last decades, we and others have demonstrated that the concept of heme complexation is appropriate for the development of highly active inhibitors of CYP enzymes. Furthermore, high selectivity could be obtained by modifying the corresponding molecules using ligand- and structure-based medicinal chemistry strategies. In the present study, etomidate was used as a starting point to develop highly potent and selective CYP11B1 inhibitors that were superior to the currently used drugs ketoconazole, metyrapone, and etomidate. The latter show a broad range of adverse effects, which are mainly due to inhibition of other CYPs. Therefore, selectivity studies regarding the most important steroidogenic CYPs, CYP11B2, CYP17, and CYP19, were performed.

Table 1. Inhibition of CYP11B1 and CYP11B2 by compounds **1**-**24**



1 - 12

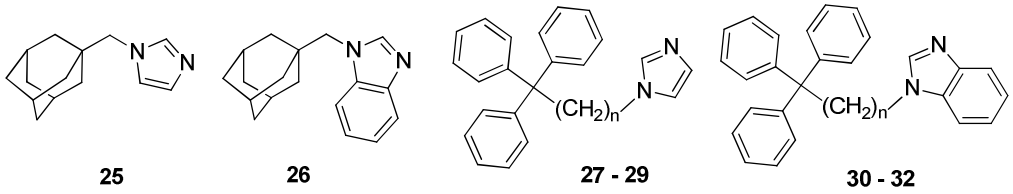


13 - 24

Structure		IC_{50} value (nM)[a,b]		sf[c]		IC_{50} (nM)[a,b]		sf[c]
R	No	CYP11B1	CYP11B2		No	CYP11B1	CYP11B2	
H	**1**	135	456	3.4	**13**	246	865	3.5
4-Cl	**2**	140	46	0.3	**14**	635	107	0.2
2-Me	**3**	61	62	1.0	**15**	779	262	0.3
3-Me	**4**	48	110	2.3	**16**	194	309	1.6
4-Me	**5**	258	320	1.2	**17**	500	520	1.0
3,5-di-Me	**6**	32	77	2.4	**18**	188	632	3.4
2,4,6-tri-Me	**7**	24	30	1.3	**19**	>1000	>1000	
2,3,4,5,6-penta-Me	**8**	5	23	4.6	**20**	>1000	>1000	
2-Ph	**9**	15	39	2.6	**21**	>1000	>1000	
3-Ph	**10**	46	265	5.8	**22**	369	2226	6.0
4-Ph	**11**	32	637	20	**23**	197	1903	10
3,5-di-Ph	**12**	128	332	2.6	**24**	>5000	>5000	
MTP[d]		15	72	4.8				
ETO[d]		0.5	0.1	0.5				
KTZ[d]		127	67	0.5				

[a] Mean value of at least three experiments. The deviations were within < ± 25 %.
[b] Hamster fibroblasts expressing human CYP11B1/CYP11B2; substrate 11-deoxycorticosterone, 100 nM
[c] sf: selectivity factor: IC_{50} (CYP11B2)/IC_{50} (CYP11B1)
[d] **MTP**: Metyrapone; **ETO**: Etomidate; **KTZ**: Ketoconazole

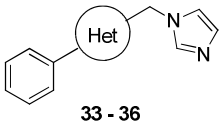
Table 2. Inhibition of CYP11B2 and CYP11B1 by compounds **25**-**32**



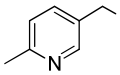
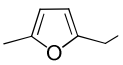
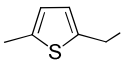
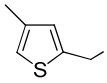
		IC_{50} value (nM)[a,b]		sf[c]		IC_{50} value (nM)[a,b]		sf[c]
n	No	CYP11B1	CYP11B2		No	CYP11B1	CYP11B2	
-	**25**	5	30	5.9	**26**	75	677	9
0	**27**	3	11	3.4	**30**	>1000	>1000	
1	**28**	4	8	2.0	**31**	>1000	>1000	
2	**29**	80	290	3.6	**32**	>1000	>1000	
MTP[d]		15	72	4.8				
ETO[d]		0.5	0.1	0.2				
KTZ[d]		127	67	0.5				

[a] Mean value of at least three experiments. The deviations were within < ± 25 %.
[b] Hamster fibroblasts expressing human CYP11B1/CYP11B2; substrate 11-deoxycorticosterone, 100 nM
[c] sf: selectivity factor: IC_{50} (CYP11B2)/IC_{50} (CYP11B1)
[d] **MTP**: Metyrapone; **ETO**: Etomidate; **KTZ**: Ketoconazole

Table 3. Inhibition of CYP11B1, CYP11B2, CYP17 and CYP19 by compounds **33**-**36**



33 - 36

	Structure	IC_{50} value (nM)[a,b]		sf[c]	inhibition (%)	
Compound	Het	CYP11B1	CYP11B2		CYP17[a,e]	CYP19[a,f]
11	-	32	637	20	40	5
33		152	2768	18	4	0
34		46	372	8.1	0	51
35		43	353	8.2	21	26
36		19	277	15	21	72
MTP [d]		15	72	4.8		
ETO [d]		0.5	0.1	0.2		
KTZ [d]		127	67	0.5		

[a] Mean value of at least three experiments. The deviations were within < ± 25 %.
[b] Hamster fibroblasts expressing human CYP11B1/CYP11B2; substrate 11-deoxycorticosterone, 100 nM
[c] sf: selectivity factor: IC_{50} (CYP11B2)/IC_{50} (CYP11B1)
[d] **MTP**: metyrapone; **ETO**: etomidate; **KTZ**: ketoconazole
[e] *E. coli* expressing human CYP17; substrate progesterone, 25 µM; inhibitor concentration 2.0 µM
[f] Human placental CYP19; substrate androstenedione, 500 nM; inhibitor concentration 500 nM

The starting point was the unsubstituted *N*-benzylimidazole **1**[29] and the corresponding benzimidazole **13**. Both showed a good inhibition of the target enzyme (IC_{50} = 135 and 246 nM) and reasonable sfs of 3.4 and 3.5 toward CYP11B2. Interestingly, the introduction of a chloro substituent into the phenyl ring led to an inversion of the selectivity and resulted in CYP11B2 inhibitors (**2** and **14**). As we hypothesized from the results of Roumen et al.,[29] the introduction of apolar substituents increased the inhibitory activity of the imidazoles as can be seen for the methyl-substituted compounds **3**–**8**. The penta methyl compound **8** was the most active compound of this series (IC_{50} = 5 nM). As a similar structure–activity relationship (SAR) was observed for inhibition of CYP11B2, only a slight enhancement of the sf was found for **8**. In contrast, increasing the number of methyl groups at the benzimidazoles was not tolerated (**19** and **20**).

Remarkable SARs were found for the phenyl-substituted compounds. In the case of the *N*-benzylimidazoles, **9**–**11** strongly inhibited cortisol formation (IC_{50} = 15–46 nM). A strong decrease of CYP11B2 inhibition was observed in the order *ortho, meta,* and *para* (**9**, IC_{50} = 39 nM; **10**, 265 nM; and **11**, 637 nM). Further phenyl substituents at the *N*-benzyl moiety of benzimidazole **13** increased activity only in the case of **23,** a fairly selective compound (sf = 10).

Phenyl substitution at the methylene spacer and its elongation resulted in a loss of activity for the benzimidazoles **30**–**32**, while the corresponding imidazoles **27**–**29** showed very high inhibition values, especially **27** (IC_{50} = 3 nM). However, these compounds are also highly potent CYP11B2 inhibitors. The replacement of the phenyl ring of **1** and **13** by an adamantane moiety led to the imidazole **25**, a highly potent (IC_{50} = 5 nM) and moderately selective (sf = 6) compound, and the corresponding benzimidazole **26,** showing a decreased activity (IC_{50} = 75 nM) but higher selectivity (sf = 9).

In the benzimidazole class, a series of highly selective compounds (**22**, **23**, and **26**) was found, demonstrating that this rigidification of the methyl ester group of etomidate was an appropriate optimization strategy. However, the compounds were less active than the imidazoles, especially in case of the bulky core compounds **24** and **30**–**32** or the *ortho*-substituted phenyl compounds **15** and **19**–**21** with hindered rotation around the methylene bridge, presumably as they are not able to properly fit into the binding pocket.

As several compounds were observed to show some residual inhibition of CYP19 and CYP17, the most selective compound regarding CYP11B2, **11** was chosen for further modification, that is, exchange of the central phenyl moiety by different heterocycles.

The compounds obtained were highly potent CYP11B1 inhibitors with selectivity toward CYP11B2. The furan **34** showed no CYP17 but CYP19 inhibition. Both thiophenes **35** and **36** inhibited CYP17 to some extent but showed, especially **36**, enhanced CYP19 inhibition. The best selectivity, comparable to **11**, was achieved by introduction of a pyridine, resulting in **33** (IC_{50} = 152 nM, sf = 18), which, most importantly, did not affect CYP19 and CYP17. Regarding its activity, this compound is comparable to ketoconazole (IC_{50} = 127 nM), which is clinically used for the treatment

of Cushing´s syndrome, but highly exceeds ketoconazole (sf = 0.5) and the other clinically used compounds metyrapone (sf = 4.8) and etomidate (sf = 0.2).

Summarizing, we have discovered the first selective CYP11B1 inhibitors described so far. We regard them as novel leads for the development of drugs for the treatment of cortisol-dependent diseases. Thus, the design strategy starting from the CYP11B2 selective etomidate was successful. While Zolle et al. described chiral etomidate derivatives with a high affinity to rat adrenal membranes as well as strong inhibition of cortisol secretion without investigating selectivity issues,[27] the compounds described in this paper were examined for selectivity toward the most crucial steroidogenic CYP enzymes, and several were found to be selective.

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3.5 Optimization of the first selective steroid-11β-hydroxylase (CYP11B1) inhibitors for the treatment of cortisol dependent diseases

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Proof is on hold until submission of patent application

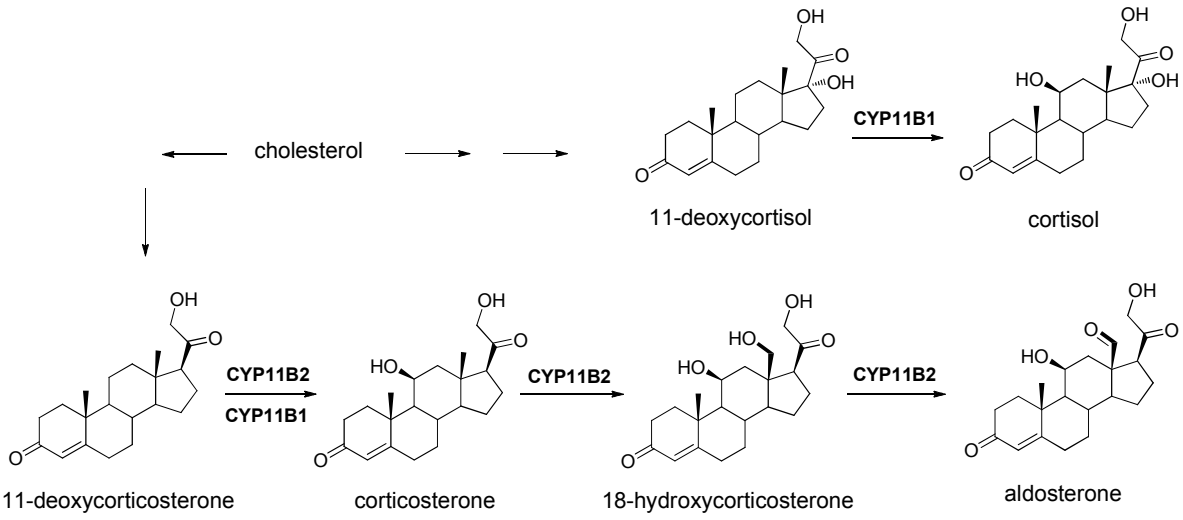
Paper V

Abstract

CYP11B1 is the key enzyme in cortisol biosynthesis and its inhibition with selective compounds is a promising strategy for the treatment of diseases associated with elevated cortisol levels like Cushing's syndrome or metabolic disease. Outgoing from a previous study from our group resulting in the first potent and rather selective inhibitor described so far (**1**, IC_{50} = 152 nM), we herein describe further optimizations of the imidazolylmethyl pyridine core. Five compounds among the 42 substances synthesized showed IC_{50} values below 50 nM. Most interesting was the naphth-1-yl compound **23** (IC_{50} = 42 nM) showing a 49-fold selectivity toward the highly homologous CYP11B2 (**1**: 18-fold) as well as selectivity toward the androgen and estrogen forming enzymes CYP17 and CYP19, respectively.

Endogenous Cushing's syndrome is a hormonal disorder caused by prolonged exposure to excessive levels of circulating glucocorticoids, therefore also called hypercortisolism. Signs and symptoms of this disorder vary, but most people develop central obesity, a round face and very often also diabetes and hypertension. Cushing's syndrome is cause of considerable morbidity and mortality. In about 80 % of all cases a pituitary hypersecretion of ACTH is observed, which is mostly related to an ACTH-secreting pituitary adenoma (Cushing's disease).[1] Benign or malignant adrenocortical tumors are the most common reasons for ACTH-independent hypercortisolism. The standard treatment for most patients is the surgical removal of the tumor or radiation therapy. However, a third of all patients are not treatable with these therapies and require temporary or permanent medication.[2] Therefore, the application of drugs lowering the elevated cortisol levels or reducing glucocorticoid activity is considered as method of choice. However, results with the glucocorticoid receptor antagonist Mifepristone show, that administration of these agents triggers a massive secretion of cortisol which is probably caused by the hypothalamic pituitary feedback mechanism.[3] A decrease of glucocorticoid formation should be a better therapeutic option. The best target for such an approach is steroid-11β-hydroxylase (CYP11B1), an adrenal CYP enzyme which directly affects cortisol production by catalyzing the hydroxylation of deoxycortisol in 11β-position (Scheme 1). There are already inhibitors of cortisol biosynthesis like Ketoconazole, Etomidate and Metyrapone in clinical use.[4] However, all of them show severe side effects due to the fact that they are unselective, i.e. they inhibit a broad range of CYP enzymes or hydroxysteroid dehydrogenases (HSDs).

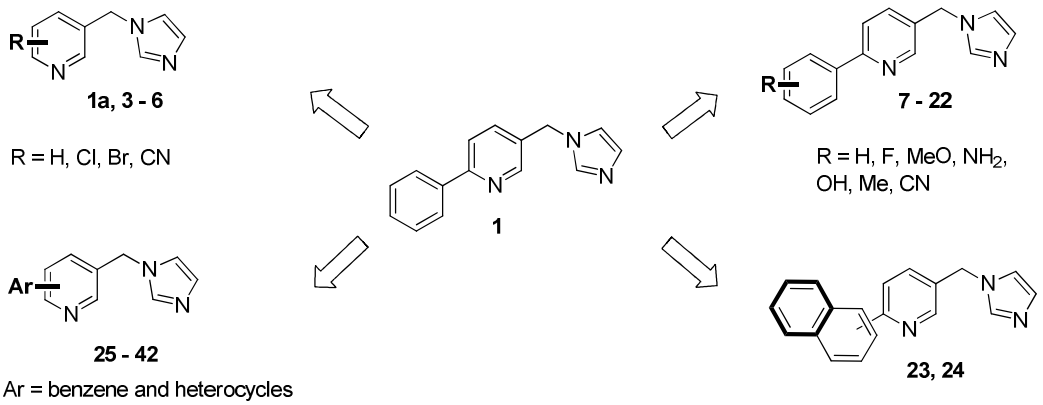
Scheme 1. Role of CYP11B1 and CYP11B2 in cortisol and aldosterone biosynthesis



A major problem in the development of CYP enzyme inhibitors is the selectivity versus other CYP enzymes. In the past we and others have successfully developed selective inhibitors of steroidogenic CYP enzymes. Aromatase (estrogen synthase, CYP19)[5-8] and 17α-hydroxylase-C17,20-lyase (CYP17)

inhibitors[9-12] are first line drugs for the treatment of breast cancer and upcoming therapeutics for castration refractory prostate cancer, respectively. In case of adrenal CYP11B enzymes matters are much more challenging as the homology between CYP11B1 and CYP11B2 (aldosterone synthase, Scheme1) is very high (93%)[13] and for a long time it was considered impossible to obtain selective inhibitors. Recently, however, we succeeded in the development of highly active and selective CYP11B2 inhibitors[14-18] with *in vivo* activity reaching selectivity factors of 1000 with regard to CYP11B1.[19,20] Moreover, we very lately discovered the first rather selective CYP11B1 inhibitors using an Etomidate-based approach.[21] The best compound identified was the imidazolylmethyl pyridine **1** (Scheme 2), which has a comparable activity to the clinically administered Ketoconazole, but strongly exceeds its selectivity. This compound showed a rather strong inhibition of the target enzyme (IC_{50} = 152 nM), a fairly good selectivity toward CYP11B2 (selectivity factor, sf = 18) and did not affect CYP17 and CYP19. For improving the activity and selectivity we describe here structural modifications of **1** leading to 42 novel compounds (Scheme 2). The phenyl ring was either replaced by small substituents (**1a**, **3** – **6**), or small groups were introduced into the phenyl ring (**7** – **22**). Furthermore, annulation of a benzene to the phenyl moiety resulted in naphthalene compounds **23** and **24**. Finally, the phenyl moiety was exchanged by several heterocycles (**25** – **42**). All compounds were tested for inhibition of human CYP11B1 and CYP11B2, the 24 most interesting compounds were selected for evaluation of CYP19 and CYP17 inhibition.

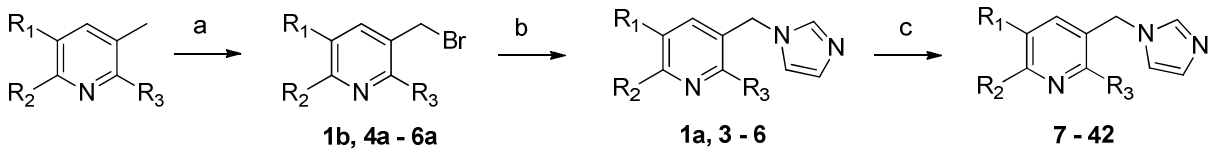
Scheme 2: Synthesized inhibitors



The synthesis of compounds **1a**, **3** – **42** is shown in Scheme 3. The reaction sequence is basically as already described[21]. The key reaction leading to the final compounds **7** – **42** was a Suzuki coupling using **1a** and compounds **5** and **6**, respectively, and the corresponding boronic acids. Compounds **5** and **6** were obtained from 3-bromo-2-chloro-5-methylpyridine and 2-bromo-3- methylpyridine, respectively. Interestingly, Suzuki coupling of **6** with phenylboronic acid and furan-2-ylboronic acid led to bis-substituted compounds **23** and **40**, while reaction with thiophen-2-ylboronic acid only

replaced the bromine, not the chlorine in **6** leading to **33**. The unsubstituted pyridines **2** and **3** were obtained via S_N reaction from the commercially available corresponding bromomethylpyridines (synthesis of **2** not shown). Compound **4** was obtained from 3-methylpicolinonitrile via Wohl-Ziegler bromination and subsequent S_N reaction with imidazole.

Scheme 3. Synthesis of compounds **1a** and **3** - **42**

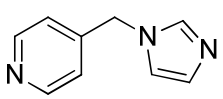


[a]Conditions: (a) **Method A**: NBS, DBPO, CCl_4, 90°C, 12h; (b) **Method B:** imidazole, K_2CO_3, acetonitrile, 90°C, 2 h; (c) **Method C:** boronic acid, $Pd(PPh_3)_4$, Na_2CO_3, toluene/ MeOH/ H_2O, reflux, 5 h.

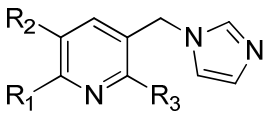
For the determination of CYP11B1 and CYP11B2 inhibition V79 MZh cells expressing either human CYP11B1 or CYP11B2 were used and [^{3}H]-labeled 11-deoxycorticosterone as substrate.[22,23] Metyrapone, Etomidate, Ketoconazole and **1** served as references. Compounds **2** and **3**, bearing only the unsubstituted pyridine ring, showed moderate inhibitory activity toward CYP11B1 (Table 1). Introduction of different substituents into **3** led to the highly active *o*-Br compound **5** (IC_{50} = 61 nM) with a good selectivity toward CYP11B2 (sf = 15). While introduction of *o*-CN and *p*-Br was not changing activity strongly, the *p*-Cl,*m*-Br compound **6** (IC_{50} = 168 nM) showed good activity, but only moderate selectivity.

Introduction of F into **1** led to interesting results. Compound **7** with *o*-F substitution showed an increase in potency and selectivity while the *m*-F and *p*-F compounds **8** and **9** exhibited reduced inhibition compared to **1**. The *m*,*p*-di-F compound **10** showed a similar activity as the corresponding mono substituted compounds and a reduced selectivity. Introduction of *p*-OH into **8** increased activity strongly (**11**, IC_{50} = 17 nM). Regarding MeO substitution, only the *o*-MeO compound **12** showed good CYP11B1 inhibition (IC_{50} = 167 nM) and a high selectivity (sf = 26). NH_2 substitution resulted in highly active compounds, no matter in which position the group is located (**15** – **17**, IC_{50} = 101 – 110 nM). Regarding selectivity, however, differences could be observed. Compound **16** turned out to be the most selective compound (sf = 31). Introduction of a Me group into **16** strongly decreased inhibition (**18**, IC_{50} = 542 nM). Further substituents like CN (**19**, **20**), formyl (**21**) and diphenylamino (**22**) did not enhance the potency of **1** (IC_{50} > 200 nM).

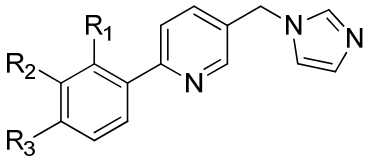
Table 1. Inhibition of CYP11B2 and CYP11B1 by compounds **2 - 22**



2



1a, 3 - 6



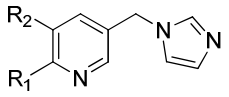
1, 7 - 22

No.	Structure			IC_{50} value (nM)[a,b]		sf [c]
	R_1	R_2	R_3	CYP11B1	CYP11B2	
2				663	>1000	
3				816	>1000	
4			CN	971	>1000	
1a	Br			500	>1000	
5			Br	61	911	15
6	Cl	Br		168	576	3.4
7	F			72	1736	24
8		F		320	>1000	
9			F	213	2153	10
10		F	F	329	1665	5
11		F	OH	17	237	14
12	MeO			167	4391	26
13			MeO	782	>1000	
14		MeO	MeO	>1000	>1000	
15	NH_2			101	2114	21
16		NH_2		110	3407	31
17			NH_2	106	528	5
18		NH_2	Me	542	>1000	
19		CN		409	>1000	
20			CN	782	>1000	
21			CHO	246	>1000	
22			di-Ph-N	611	n.i.[d]	
1[e]				152	2768	18
MTP [f]				15	72	4.8
ETO [f]				0.5	0.1	0.2
KTZ [f]				127	67	0.5

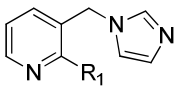
[a] Mean value of at least three experiments. The deviations were within < ± 25 %.
[b] Hamster fibroblasts expressing human CYP11B1 or CYP11B2; substrate 11-deoxycorticosterone, 100 nM
[c] sf: selectivity factor: IC_{50} (CYP11B2)/IC_{50} (CYP11B1)
[d] n.i.: no inhibition at an inhibitor concentration of 500 nM
[e] from Ref. 21
[f] **MTP**: Metyrapone; **ETO**: Etomidate; **KTZ**: Ketoconazole

Annulation of an additional benzene ring yielding the naphthalenes **23** and **24** (Table 2) resulted in a remarkable finding. In case of the 1-naphthyl compound **23** a strong enhancement of activity and selectivity was observed (IC_{50} = 42 nM, sf = 49). In contrast, the 2-naphthyl compound **24** showed only moderate activity and low selectivity (IC_{50} = 246 nM, sf = 3).

Table 2. Inhibition of CYP11B2 and CYP11B1 by compounds **23 - 42**



V/1, V/23 - V/35, V/37 -V/42



V/36

No.	Structure		IC_{50} value (nM)[a,b]		sf [c]
	R_1	R_2	CYP11B1	CYP11B2	
23	1-naphthalene		42	2075	49
24	2-naphthalene		246	782	3.2
25	Ph	Ph	362	851	2.4
26	3-pyridine		502	3955	8
27	4-pyridine		139	487	3.5
28	5-pyrimidine		971	n.i.[d]	
29	3-(6-methoxypyridine)		>1000	>1000	
30	4-isoquinoline		95	914	10
31	2-thiophene		75	1243	17
32	3-thiophene		126	3265	26
33	2-(5-chlorothiophene)		362	929	2.6
34	2-(5-formylthiophene)		62	968	16
35	Cl	2-thiophene	73	416	6
36	2-thiophene		16	251	16
37	2-benzo[*b*]thiophene		269	281	1.0
38	3-benzo[*b*]thiophene		40	1157	29
39	2-furan		167	5159	31
40	3-furan		76	2832	37
41	2-benzo[*b*]furan		500	>1000	
42	2-furan	2-furan	29	830	29
1[e]	Ph		152	2768	18
MTP [f]			15	72	4.8
ETO [f]			0.5	0.1	0.2
KTZ [f]			127	67	0.5

[a] Mean value of at least three experiments. The deviations were within < ± 25 %.
[b] Hamster fibroblasts expressing human CYP11B1 or CYP11B2; substrate 11-deoxycorticosterone, 100 nM
[c] sf: selectivity factor: IC_{50} (CYP11B2)/IC_{50} (CYP11B1)
[d] n.i.: no inhibition at an inhibitor concentration of 500 nM
[e] from Ref. 21
[f] **MTP**: Metyrapone; **ETO**: Etomidate; **KTZ**: Ketoconazole

Exchange of the phenyl moiety of **1** by nitrogen containing heterocycles (**26** – **30**) led only in case of 4-pyridine (**27**, IC_{50} = 139 nM) and 4- isoquinoline (**30**, IC_{50} = 95 nM) to fairly active compounds, however, showing only poor selectivity. Contrarily, thiophene containing compounds were in most cases highly active, e.g. the 2-thiophene **31** (IC_{50} = 75 nM). Introduction of 5-Cl or 5-formyl into the thiophene ring of **31** reduced activity or did not change it (**33**, **34**). The 3-thiophene **32** (IC_{50} = 126) was less active than its 2-isomer **31**. Interestingly, annulation of a benzene ring onto the thiophene moiety of compound **31** and **32** led to reverse effects. In case of **31**, the activity was reduced whereas for compound **32** it was increased (**37**, IC_{50} = 269 nM, **38**, IC_{50} = 40 nM). The displacement of the 2-thiophene group of **31** into other positions did not change activity in case of **35** (bearing an additional

Cl-substituent) while in **36** potency was strongly increased (IC_{50} = 16 nM). Contrariwise to the thiophenes, the CYP11B1 inhibition was somewhat higher for the 3-furan **40** (IC_{50} = 76 nM) compared to the 2-furan **39** (IC_{50} = 167 nM). As seen with the 2-thiophene **31** annulation of a benzene ring onto the 2-furan ring of **39** decreased activity (**41**, IC_{50} = 500 nM).

The introduction of an additional ring, phenyl into compound **1** and 2-furanyl into compound **39** again led to opposed results: Compound **25** (IC_{50} = 362 nM) showed a somewhat reduced activity compared to the parent compound whereas compound **42** (IC_{50} = 29 nM) turned out to be much more potent than **39**.

Inhibition of CYP17 was investigated using the 50,000 g sediment of the E. coli homogenate recombinantly expressing human CYP17 and progesterone (25 µM) as substrate.[11,24] The compounds were tested at a concentration of 2.0 µM. All investigated substances showed no effect (inhibition values <10%, data not shown). Inhibition of CYP19 was determined with human placental microsomes and [1β-^{3}H]androstenedione as substrate at an inhibitor concentration of 500 nM as described.[25] Only **33** and **34** exhibited little inhibition (30 and 19 %, respectively), while all other substances showed no inhibition (values < 10 %, data not shown).

Summarizing, we succeeded in the further optimization of our recently discovered lead compound **1**. The activity was enhanced and a number of inhibitors showing IC_{50} values below 50 nM were found. Furthermore, the selectivity toward the highly homologous CYP11B2 was increased. As expected, most of the compounds exhibited no inhibition of CYP17 and CYP19. Interestingly, already small compounds like the bromo substituted **5** exhibited high inhibitory activity toward the target enzyme, while being highly selective toward the other CYPs. Via introduction of different substituents at the phenyl ring of **1**, we were able to enhance the selectivity up to 31. In this series we observed, that substituents in *ortho* position resulting in non-planar compounds (**7**, **12**, **15**) had a beneficial effect. Contrarily, planarity seems to be crucial for a high inhibition of CYP11B2. As a consequence of this, *ortho* substituted compounds showed a high selectivity. It is striking that H-bond donors in *para* position of the phenyl ring enhanced the activity (OH, **11**; NH_2, **17**), while all other *para* substituents lowered the inhibitory potency compared to **1**. The replacement of the phenyl ring of **1** by different heterocycles and their introduction into different positions of the central pyridine led to compounds with selectivities up to 37. In this series, the introduction of thiophene and furan led to highly active compounds with interesting SARs regarding selectivity. The furans **37** and **38** were observed to be more selective toward CYP11B2 than their corresponding thiophenes **29** and **30**. This might be due to the less aromatic character of the furan, which seems to fit better into the CYP11B1 binding site than in the binding pocket of CYP11B2. Additionally, the position of the heteroatom plays a role for selectivity. For the furans and the thiophenes the 3-position is preferred. The finding observed

regarding the annulation of a benzene nucleus to **1** and **36** leading to **23** and **24** as well as **37** and **38**, respectively, is supportive of the above mentioned hypothesis that non-planarity is enhancing selectivity, as the compounds with a hindered rotation around the aryl-aryl bond (**23** and **38**) show much higher selectivities than their free rotatable isomers.

One of the most potent compounds of this series, similarly active as the clinically used Metyrapone, is **23**. However, in contrast to Metyrapone, **23** is much more selective: it is the most selective compound described so far and should be a promising candidate for further development. A potent and selective CYP11B1 inhibitor as **23** might not only be a good therapeutic for the treatment of Cushing's syndrome, it might also be beneficial for treating metabolic syndrome as elevated cortisol levels also appear to play a central role in this disease.[26] A phase IIa study using the 2*S*,4*R* enantiomer of Ketoconazole (DIO-902) with patients suffering from type 2 diabetes and other components of metabolic syndrome showed encouraging results. Therein it has been shown, that administration of this drug resulted in reduced levels of HbA1c, cholesterol as well as weight loss and decreased blood pressure, all parameters known to be associated with metabolic disease.[27,28]

Supporting Information Available: Synthetic experimental details, analytical data of compounds and biological assay protocols. This material is available free of charge via the Internet at http://pubs.acs.org.

Acknowledgement

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4 Diskussion und Ausblick

4.1 Entwicklung neuer nichtsteroidaler CYP17 Inhibitoren

Das Ziel der vorliegenden Arbeit war die Entwicklung neuer nichtsteroidaler CYP17 Inhibitoren ausgehend von biphenylischen Leitstrukturen, die in unserer Arbeitsgruppe entwickelt wurden. Dabei sollte ein Meilenstein die gezielte Erhöhung der Aktivität dieser Verbindungen darstellen und in einem nächsten Schritt war das Ziel die sukzessive Verbesserung des Selektivitätsprofils dieser Verbindungen.

Eine Kombination aus liganden- und strukturbasiertem Ansatz lieferte die ersten neuen Strukturen, die im Kapitel 3.1. beschrieben wurden.

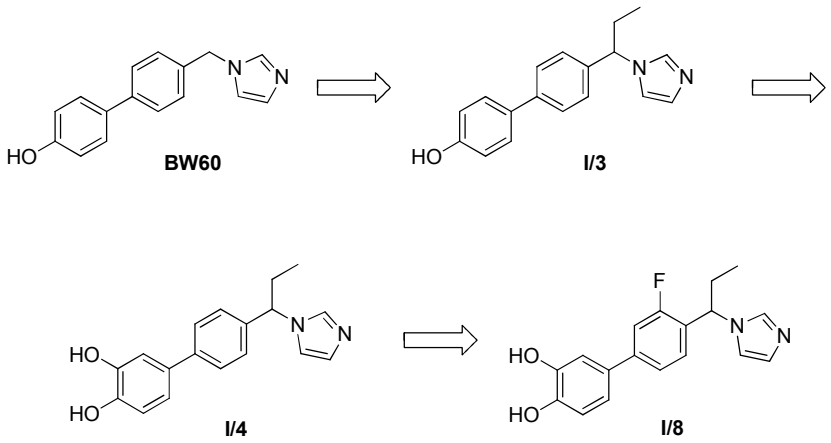


Abbildung 5: Optimierung neuer CYP17 Inhibitoren

Die Leitstruktur hierfür stellte der in unserer Arbeitsgruppe entwickelte CYP17 Inhibitor **BW60** (IC_{50} 310 nM) (Abb.5) dar.[33] Diese biphenylische Verbindung wurde durch Simplifizierung der Struktur des endogenen CYP17-Substrates Pregnenolon erhalten und ist ein sogenanntes A-C-Ring-Mimetikum. Da ein Docking dieser Verbindung in unser CYP17 Modell eine kleine hydrophobe Tasche in unmittelbarer Nähe der Methylenbrücke zeigte, wurden zunächst die kleinen, lipohilen Reste Methyl und Ethyl in dieser Position eingeführt. Durch Substitution mit Ethyl (**I/3**, IC_{50} = 231 nM) konnte bereits eine starke Verbesserung der inhibitorischen Aktivität der Ausgangsverbindung erzielt werden. Da Pregnenolon, neben Progesteron das endogene Substrat von CYP17, eine Hydroxy-Gruppe am A-Ring trägt und somit in dieser Region hydrophile Interaktionen in der Bindetasche zu erwarten sind, wurden in einem nächsten Schritt weitere Hydroxy-Gruppen in verschiedenen Positionen des A-Rings eingeführt (**I/3** – **I/6**). Hierbei zeigte sich, dass die höchste inhibitorische Aktivität durch einen zweiten

Hydroxy-Substituenten in Position 3 des A-Rings (**I/5**, IC_{50} = 152 nM) erreicht werden konnte. Die Hemmaktivität dieser beiden hochpotenten Verbindungen konnte durch die Einführung eines Fluor-Substituenten im C-Ring noch verstärkt werden und man erhielt die beiden besten Verbindungen dieser Studie: Die einfach hydroxylierte Verbindung **I/7** (IC_{50} = 90 nM) und **I/8** (IC_{50} = 52 nM) mit einer weiteren vicinalen Hydroxy-Gruppe, die wie zu erwarten nochmals eine gesteigerte Hemmaktivität zeigte. Diese beiden Verbindungen zeigen damit eine deutlich stärkere CYP17 Hemmung als die Referenz Ketoconazol und erreichen sogar die Potenz von Abirateron. Ein Nachteil dieser Verbindungen ist jedoch die relative hohe Hemmung des hepatischen CYP3A4 (>88 % bei 10µM) und des Hauptenzyms der Glucocorticoid-Biosynthese. Darum wurde eine weitere Optimierung der gefundenen Verbindungen durchgeführt.

In einer folgenden Serie von Verbindungen (Kapitel 3.2) wurde deshalb der A-Ring durch Annelierung eines weiteren Rings zum Naphthalin erweitert und auch hier ein Methoxy- oder Hydroxy-Rest substituiert. Eine weitere Untersuchung der Struktur-Aktivitäts-Beziehungen erfolgte durch Betrachtung unterschiedlicher Substitutionsmuster der Ethylgruppe an der Methylenbrücke. Die beste Verbindung dieser Serie, **II/21**, die neben einer Hydroxy-Gruppe am Naphthalin noch eine zweite Ethyl-Gruppe am Linker trägt, ist zwar mit einem IC_{50} von 591 nM weniger aktiv als die Ausgangsverbindung dieser Optimierungsstudie, jedoch immer noch deutlich aktiver als das klinisch genutzte Ketoconazol. Mit dieser Verbindung ist es außerdem erstmals gelungen, die Hemmung des hepatischen Enzyms CYP3A4 stark herabzusenken (64 % bei 10 µM).

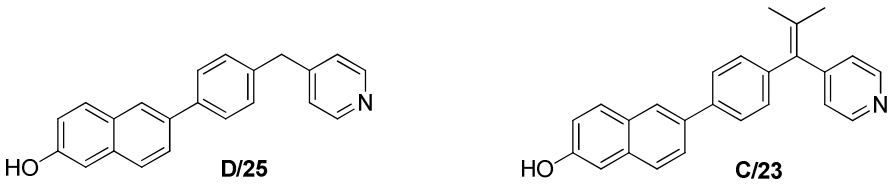


Abbildung 6: Die optimierten CYP17-Inhibitoren **D/25** und **C/23**

Zuletzt erfolgte noch ein Austausch des Imidazol-Rings gegen 4-Pyridin. Die aus dieser Serie (Abb.6) resultierende Verbindung **C/23** stellt mit einem IC_{50} Wert von 62 nM einen hochaktiven CYP17 Hemmstoff dar. Vergleicht man **C/23**, die einen *i*-Propyliden-Linker an der Methylenbrücke trägt, mit der korrespondierenden **D/25**, wird klar, dass die Einführung dieses kleinen Restes sehr große Auswirkungen auf die Aktivität und auch die Selektivität dieser Verbindungsklasse hat. Zeigt **D/25** lediglich einen IC_{50} von 438 nM, führt die Einführung des *i*-Propyliden-Restes zu einer 7-fachen Steigerung der Aktivität. Zusätzlich konnte die Hemmung der weiteren steroidogenen CYP Enzyme CYP11B1 und CYP11B2, die bei der Verbindung **D/25** noch deutlich vorhanden war (IC_{50} (B1) = 627 nM, IC_{50} (B2) = 1130 nM), mit Einführung der *i*-Propyliden-Gruppe stark reduziert werden. Verbindung **C/23**, die für beide CYP11B-Enzyme IC_{50}-Werte größer 5000 nM zeigt und noch dazu

selektiv gegenüber Aromatase (IC_{50} = 48700 nM) ist, kann damit als einer der bislang aktivsten und selektivsten CYP17 Hemmstoffe unseres Arbeitskreises angesehen werden. Angesichts der gemessenen *in vitro* Daten übertrifft diese nichtsteroidale Substanz sogar das in klinischer Studie befindliche Abirateron und hat somit das Potenzial zur Weiterentwicklung. Um eine perorale Verabreichung dieser Substanz zu gewährleisten, könnte ein Pro-Drug-Konzept zur Schützung der Hydroxy-Gruppe nötig sein.

4.2 Entwicklung neuer nichtsteroidaler CYP Inhibitoren durch Kombination bekannter Strukturen

Da in unserem Arbeitskreis seit langem erfolgreiche Konzepte zur Hemmung der CYP Enzyme der Steroidbiosynthese durchgeführt werden, diente der im Kapitel 3.3 beschriebene Ansatz zur Auffindung neuer Leitstrukturen.

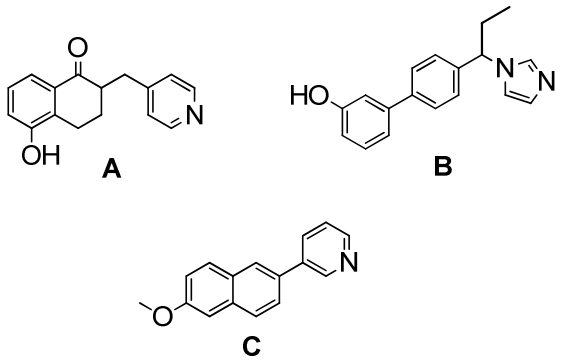
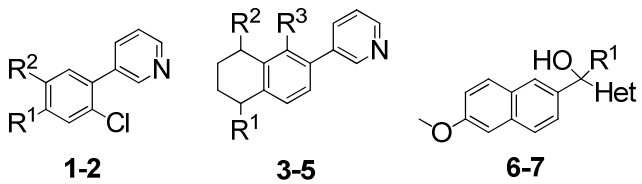


Abbildung 7: Hochaktive Inhibitoren von CYP19 (**A**), CYP17 (**B**) und CYP11B2 (**C**)

Ausgehend von den hochaktiven Inhibitoren A, B und C (Abb.7) wurde durch eine neue Kombination ihrer Grundstruktur, des Heterozyklus zur Stickstoffkomplexierung und ihrer Verbrückung eine Serie neuer Verbindungen dargestellt (Tab.1).

Tabelle 1 . Verbindungen **III/1**-**III/7**.



Verbindung	R^1	R^2	R^3	Het
III/1	2-OMe-Ph	H		
III/2	H	3-OMe-Ph		
III/3	=O	H	Cl	
III/4	H	=O	H	
III/5	H	OH	H	
III/6	Et			3-Py
III/7	H			4-Py

Bezüglich des Grundgerüstes können die Verbindungen in Biphenyle (**III/1** und **III/2**), Tetrahydronaphthaline (**III/3** – **III/5**) und in Naphthaline (**III/6** und **III/7**) eingeteilt werden. Während die

Grundgerüste der Verbindungen **III/1** – **III/5** direkt mit dem Heterozyklus verbunden sind, ist in den Verbindungen **III/6** und **III/7** eine Hydroxy-substituierte Methylenbrücke zwischengelagert. Diese trägt im Falle von Verbindung **III/7** einen zusätzlichen Ethyl-Rest.

Die Verbindungen **III/1** und **III/2**, die aus biphenylischen CYP17 Inhibitoren entwickelt wurden, zeigten nur sehr geringe Hemmungen der Target Enzyme. Daraus lässt sich schließen, dass in der Klasse der Biphenyle, die Art und Orientierung ihres Heterozyklus, ebenso wie die zwischengelagerte Methylenbrücke eine essentielle Rolle spielen.

In der Klasse der Naphthaline hingegen, konnte eine hochaktive Verbindung (**III/7**) gefunden werden. Ausgehend von Verbindung **C**, einem effektiven CYP11B2 Inhibitor, wurden bei Verbindungen **III/6** und **III/7** interessante SAR gefunden: Die Kombination des Naphthalin-Grundgerüstes mit einer Hydroxy-substituierten Brücke zu einem 4-Pyridin erhielt die CYP11B2-Hemmung (IC_{50} = 140 nM) und führte zu einer moderaten CYP11B1-Selektivität (sf = 3) sowie Selektivität gegenüber CYP17 und CYP19 (IC_{50} > 1000 nM). Dagegen führte die Einführung einer Ethyl-Gruppe am Methylen-Linker und die Verschiebung des Pyridin-Stickstoffs in die 3-Position zu einer Verbindung, die lediglich noch eine schwache CYP11B1-Hemmung (IC_{50} = 491 nM) zeigte.

Sehr interessante Ergebnisse konnten in der Klasse der Tetralone gefunden werden. Verbindung **III/3**, entwickelt durch neue Verknüpfung zwischen Grundgerüst und Heterozyklus aus dem Aromatase Inhibitor **A**, zeigte erwartungsgemäß eine hohe CYP19-Hemmung (IC_{50} = 19 nM). Zusätzlich stellte sie aber mit einem IC_{50} von 10 nM den aktivsten CYP11B2-Hemmstoff dieser Serie dar und kann somit als attraktiver Startpunkt zur Entwicklung dualer Inhibitoren dieser beiden Enzyme betrachtet werden. Im Gegensatz dazu verloren das Tetralon **III/4** und sein korrespondierender Alkohol **III/5** ihre inhibitorische Aktivität an Aromatase völlig (IC_{50} > 1000 nM), zeigen aber dafür eine hohe Hemmung von CYP11B2 (IC_{50} = 114 und 100 nM). Damit stellen diese Verbindungen hochaktive und selektive CYP11B2 Inhibitoren dar.

Zusammenfassend lässt sich sagen, dass der vorgestellte Ansatz zur Entwicklung neuer Hit-Verbindungen als Hemmstoffe der Steroidbiosynthese erfolgreich war. So konnte ausgehend von dem Aromatase Inhibitor **A** eine neue Lead-Struktur zur Entwicklung von CYP11B2-Inhibitoren gefunden werden und ein neuartiger dualer Inhibitor wurde entdeckt. Diese Verbindungen sollten nun weitergehend untersucht und optimiert werden.

4.3 Entdeckung neuer nichtsteroidaler CYP11B1 Inhibitoren und ihre Optimierung

Ein relativ neues Target, das in unserem Arbeitskreis bearbeitet wurde, stellt CYP11B1, die Cortisol-Synthase dar. Ihre selektive Hemmung kann als Meilenstein in der Therapie cortisolabhängiger Krankheiten betrachtet werden, da bislang nur unselektive Hemmstoffe, wie Etomidat, Metyrapon oder Ketoconazol auf dem Markt sind, deren Applikation mit einer Reihe schwerwiegender Nebenwirkungen verbunden ist.

Die erste Serie selektiver CYP11B1 Inhibitoren wurde im Kapitel 3.4 beschrieben. Die dort dargestellten Verbindungen wurden ausgehend von dem hochaktiven, jedoch sogar leicht CYP11B2-selektiven CYP11B1-Inhibitor Etomidat entwickelt.

Wie in Abb.8 dargestellt, wurde hierfür zunächst die Methylgruppe an der Methylenbrücke entfernt, da diese verantwortlich für die hypnotische Aktivität,[34] eine für unsere Zwecke unerwünschte Wirkung, ist. Als positiver Nebeneffekt ist die so entstandene Verbindung nicht mehr chiral. Desweiteren wurde die Ethyl-Ester-Gruppe einerseits entfernt und andererseits durch Annelierung eines rigiden Benzolkernes ersetzt. In die so entstandenen Grundstrukturen wurden nun sukzessive Methyl-, Chlor- oder Phenyl-Reste in verschiedenen Positionen des Phenylrings und der Methylgruppe sowie in unterschiedlicher Anzahl eingefügt.

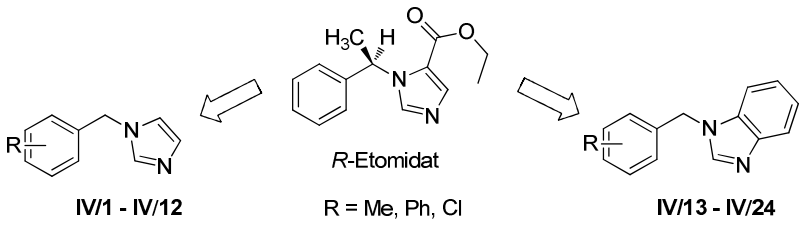


Abbildung 8: Design-Strategie zur Entwicklung selektiver CYP11B1 Inhibitoren.

Ausgangspunkt der Studie waren folglich das unsubstituierte *N*-Benzylimidazol **IV/1** und das korrespondierende Benzimidazol-Derivat **IV/13**. Beide zeigten bereits eine gute Hemmung des Targetenzyms (IC_{50} = 135 und 246 nM) und vielversprechende Selektivitätsfaktoren von 3.4 und 3.5 gegenüber CYP11B2. Wie auch schon von Roumen *et al.* gezeigt, führten Chlor-Substituenten am Phenylring zu den CYP11B2 selektiven Verbindungen **IV/2** und **IV/13**.[35] Da in ihrer Studie eine Reihe von Verbindungen mit hydrophilen Substituenten eher selektive CYP11B2-Inhibitoren darstellen, sollte in unserer Studie die Hypothese bestätigt werden, dass eine hohe inhibitorische Aktivität gegenüber CYP11B1 eher lipophile Strukturen verlangt. Daher wurden Methylgruppen in unterschiedlicher Position und Anzahl in den Phenylring eingefügt (**IV/3** – **IV/8**). Eine erste

Bestätigung für diese Therorie war die Tatsache, dass die Verbindung mit 5 Methylgruppen (**IV/8,** Abb.9) auch die aktivste (IC_{50} = 5 nM) dieser Serie darstellte. Allerdings zeigte diese Verbindung auch die vergleichsweise höchste CYP11B2-Hemmung, so dass nur eine geringe Steigerung des Selektivitätsfaktors erzielt werden konnte.

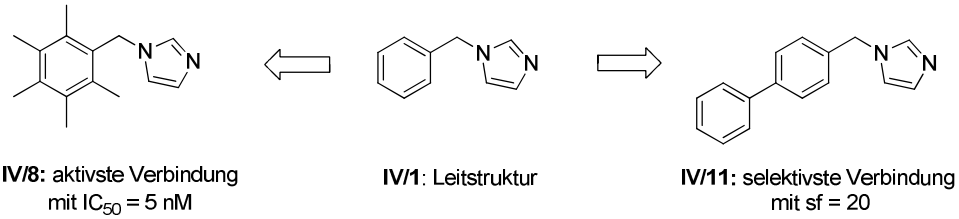


Abbildung 9: von der Leitstruktur zur aktivsten und selektivsten Verbindung

Desweiteren wurde der Effekt von Phenyl-Substituenten untersucht und führte zu interessanten Struktur-Aktivitäts-Beziehungen: Während ein solcher Substituent im Falle der *N*-Benzylimidazole zu Verbindungen (**IV/9,** *ortho*-Ph; **IV/10,** *meta*-Ph und **IV/11,** *para*-Ph) mit sehr starker Hemmung von CYP11B1 (IC_{50} = 15 – 46 nM) führte, wurden unterschiedliche inhibitorische Effekte auf CYP11B2 beobachtet und **IV/11** (sf = 20) stellte sich schliesslich als selektivste Verbindung dieser Studie heraus. Interessanterweise führte hier aber die Einführung eines zweiten Phenylrings zu einer Erniedrigung der CYP11B1 Hemmung (**IV/12**, IC_{50} = 128 nM). Im Falle der Benzimidazole zeigte nur die *para*-Phenyl-Verbindung **IV/23** eine gute Aktivität (IC_{50} = 197 nM) und auch eine hohe Selektivität (sf = 10).

Phenyl-Substitution an der Methylen-Brücke sowie ihre Verlängerung führte im Falle der Benzimidazole zu inaktiven Verbindungen (**IV/30** – **IV/32,** Abb. 10). Dagegen konnten durch diese Veränderungen bei den korrespondierenden Imidazolen **IV/27** – **IV/29** sehr hohe Aktivitäten erzielt werden. In dieser Serie ist die Verbindung **IV/27** mit dem sehr guten IC_{50} von 3 nM hervorzuheben. Leider sind diese Verbindungungen auch starke CYP11B2 Inhibitoren und somit wenig selektiv. Ein Austausch des Phenylrings der Verbindungen **IV/1** und **IV/13** gegen Adamantan führte zu dem Imidazol **IV/25**, einer hochpotenten (IC_{50} = 5 nM) und moderat selektiven (sf = 6) Verbindung und dem korrespondierenden Benzimidazol **IV/26** mit einer etwas geringeren inhibitorischen Aktivität (IC_{50} = 75 nM), dafür aber einer gesteigerten Selektivität (sf = 9).

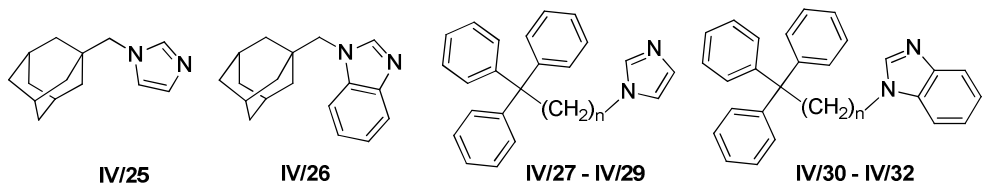


Abbildung 10: Verschiedene Variationen der Grundstruktur und Methylenbrücke

Insgesamt konnten in der Klasse der Benzimidazole also eine ganze Reihe an Verbindungen (**IV/22**, **IV/23**, **IV/26**) mit einer hohen Selektivität gegenüber CYP11B2 gefunden werden, was zeigt, dass die Rigidisierung der Methyl-Ester-Gruppe des Etomidats eine gute Optimierungsstrategie darstellte. Die Benzimidazole, insbesondere jene mit hohem sterischen Anspruch oder *ortho*-Substitution, waren jedoch weniger aktiv als die korrespondierenden Imidazole. Deshalb wurde schließlich von einer weiteren Optimierung der Benzimidazole abgesehen und der Fokus auf die vielversprechende Kleasse der Imidazol Verbindungen gesetzt.

Weil eine Reihe von Verbindungen noch eine Hemmung der Enzyme CYP17 und CYP19 zeigten, wurde die bis dahin CYP11B2-selektivste Verbindung **IV/11** weiter optimiert (Abb.11). Dazu wurde der zentrale Phenyl-Ring gegen verschiedene Heterozyklen wie Pyridin, Thiophen oder Furan ausgetauscht. Die so entstandenen Verbindungen stellten sich als hochaktive CYP11B1-Inhibitoren mit Selektivität gegenüber CYP11B2 heraus. Die beste Selektivität, vergleichbar mit der Ausgangsverbindung **IV/11**, konnte durch die Einführung eines Pyridins erreicht werden. Die so erhaltene Verbindung **IV/33** zeigte keine Hemmung der Enzyme CYP17 und CYP19 mehr und ihre Aktivität (IC_{50} = 152 nM) ist vergleichbar mit der des klinisch zur Therapie des Cushing Syndromes eingesetzten Ketoconazols (IC_{50} = 127 nM). Betrachtet man ihre CYP11B2-Selektivität (sf = 18), stellt diese Verbindung eine wesentliche Optimierung gegenüber den derzeit in der klinischen Therapie genutzten Arzneistoffen Ketoconazol (sf = 0.5), Metyrapon (sf = 4.8) und Etomidat (sf = 0.2) dar.

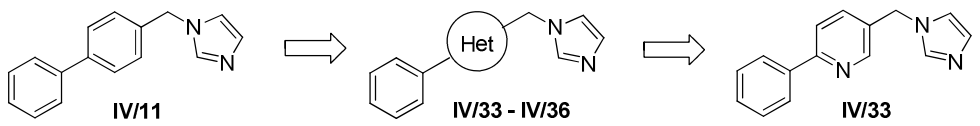


Abbildung 11. Optimierung der Verbindung **IV/11**

In einem folgenden Ansatz (Kapitel 3.5) sollte also die Leitverbindung **IV/33** hinsichtlich ihrer Aktivität und Selektivität weiter optimiert werden. Dazu wurde der Phenylring der Leitverbindung eliminiert (Abb.12), was leider zu den relativ wenig aktiven Verbindungen **V/2** und **V/3** führte und weiterhin wurden verschiedene Substituenten wie Cl-, Br- oder CN- am Pyridinring eingeführt, wodurch die hochaktive *ortho*-Br-Verbindung **V/5** (IC_{50} = 61 nM) erhalten wurde. Diese zeigt trotz ihrer vergleichsweise simplen Struktur einen vielversprechenden Selektivitätsfaktor von 15. In weiteren Schritten wurden Substituenten am Phenylring der Leitverbindung **IV/33** eingeführt und weitere Phenylringe anneliert. Außerdem wurde der Phenylring der Leitverbindung durch Heterozyklen ersetzt.

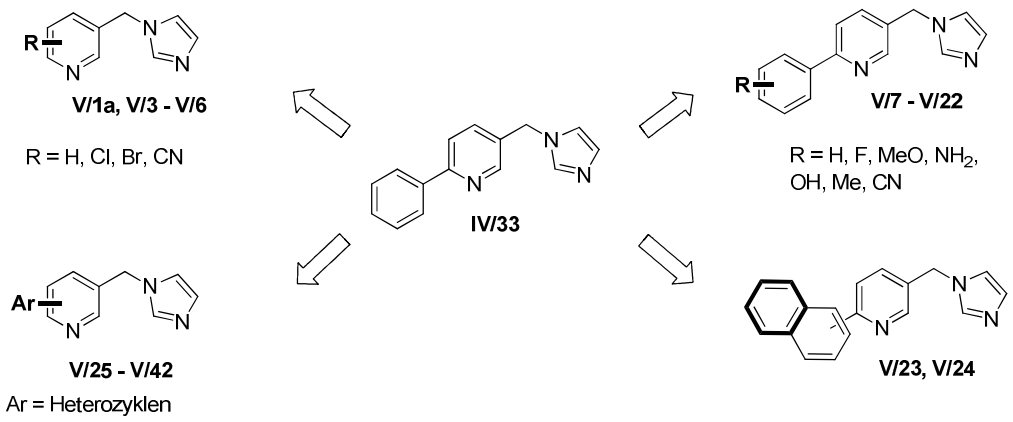
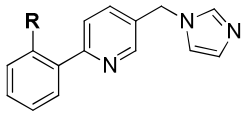


Abbildung 12: Entwicklung neuer selektiver CYP11B1-Inhibitoren aus der Lead-Verbindung **IV/33**

Wie erwartet zeigten fast alle Zielverbindungen keine Hemmung der Enzyme CYP17 und CYP19. Zunächst sei die Einführung diverser Substituenten in verschiedenen Positionen und in unterschiedlicher Anzahl am Phenylring von Verbindung **IV/33** betrachtet. Dabei konnte gezeigt werden, dass Substituenten in *ortho*-Position zur Verbrückung zum Pyridinring von Vorteil sind (Abb.13). Sie zeigten nicht nur durchweg hohe Aktivitäten, sondern lieferten auch die besten Selektivitäten dieser Serie. Zusätzlich sollte noch Verbindung **V/11** Erwähnung finden, die mit einem Hydroxy-Substituenten in *para*-Position die höchste Aktivität (IC_{50} = 17 nM) dieser Studie zeigte.



V/7: R = F, IC_{50} = 72 nM, sf = 24
V/12: R = MeO, IC_{50} = 167 nM, sf = 26
V/15: R = NH_2, IC_{50} = 101 nM, sf = 21

Abbildung 13: *Ortho*-Substituenten führen zu hohen Aktivitäten und guten Selektivitäten

Auch die Erweiterung des Phenylrings der Leitverbindung **IV/33** durch Annelierung eines weiteren Benzolkernes lieferte bemerkenswerte Ergebnisse (Abb. 14). Im Fall der 1-Naphthyl-Verbindung **V/23** konnten sowohl Aktivität als auch Selektivität stark verbessert werden (IC_{50} = 42 nM, sf = 49). Im Gegensatz dazu ist die korrespondierende 2-Naphthyl-Verbindung **V/24** nur moderat aktiv (IC_{50} = 246 nM) und wenig selektiv (sf = 3).

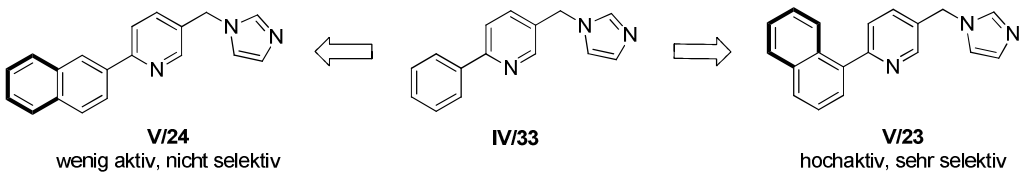
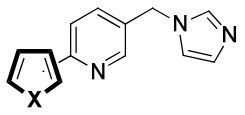


Abbildung 14: Entwicklung der Naphthyl-Verbindungen **V/23** und **V/24**

In einem weiteren Optimierungsschritt wurde der Phenylring von Verbindung **IV/33** gegen eine Reihe unterschiedlicher Heterozyklen ausgetauscht. Bei den stickstoffhaltigen Heterozyklen (**V/26** – **V/30**) konnten hier zwei potente Verbindungen gefunden werden: Das 4-Pyridin **V/27** (IC_{50} = 139 nM) und das 4-Isochinolin **V/30** (IC_{50} = 95 nM). Beide zeigten allerdings nur eine schwache Selektivität.

Dagegen waren die thiophen- und furanhaltigen Inhibitoren (Abb.15) zumeist hochaktiv und selektiv. Interessanterweise stellte sich beim Vergleich des 2-Furans **V/39** (IC_{50} = 167 nM) mit dem entsprechenden 3-Furan **V/40** (IC_{50} = 76 nM) ein umgekehrtes Verhältnis bezüglich der inhibitorischen Aktivität im Vergleich zu den korrespondierenden Thiophenen **V/31** und **V/32** heraus. Bei Betrachtung der Selektivitäten fällt auf, dass die Furane insgesamt selektiver als die thiophenhaltigen Inhibitoren sind. Innerhalb der beiden Klassen lässt sich noch beobachten, dass die an Position 3 im Vergleich zu den an Position 2 verbrückten Verbindungen deutlich selektiver sind. Somit ergibt sich das 3-Furan **V/40** mit einem sf von 37 als selektivste Verbindung dieser Serie.



V/31: Het = 2-Thiophen, IC_{50} = 75 nM, sf = 17
V/32: Het = 3-Thiophen, IC_{50} = 126 nM, sf = 26
V/39: Het = 2-Furan, IC_{50} = 167 nM, sf = 31
V/40: Het = 3-Furan, IC_{50} = 76 nM, sf = 37

Abbildung 15: Thiophen- und furanhaltige Strukturen im Vergleich

Weiterhin wurde versucht, zusätzliche Ringe zur Grundstrktur **IV/33** und auch zur modifizierten Struktur **V/39** hinzuzufügen. Dabei wurden sehr unterschiedliche Ergebnisse erhalten. **V/25**, mit zwei Phenylringen am zentralen Pyridin, zeigte eine reduzierte Hemmaktivität im Vergleich zur Ausgangsverbindung (IC_{50} = 362 nM), während sich die korrespondierende Furan-Verbindung **V/42** (IC_{50} = 29 nM) als deutlich aktiver im Vergleich zu **V/39** herausstellte.

Die Hemmungen der Enzyme CYP17 und CYP19 wurden bereits in Kapitel 3.5 beschrieben. Wie schon vermutet, zeigte keine der Verbindungen mit **IV/33**-Grundstruktur eine nennenswerte Hemmung dieser beiden Enzyme. Lediglich die beiden in der Grundstruktur veränderten Verbindungen **V/33** und **V/34** konnten an CYP19 noch eine gewisse inhibitorische Aktivität ausbilden.

Zusammenfassend lässt sich behaupten, dass eine Optimierung der vorher gefundenen Leitverbindung **IV/33** gelungen ist. Die Aktivität dieser Verbindung konnte stark gesteigert werden und eine Reihe von potenten CYP11B1-Inhibitoren mit IC_{50} Werten unter 50 nM wurden dargestellt. Durch Einführung verschiedener Substituenten am Phenylring von **IV/33** konnte die Selektivität bis 31 gesteigert werden. In dieser Reihe von Verbindungen konnte beobachtet werden, dass Substituenten in

ortho-Position, die dadurch zu nichtplanaren Verbindungen **V/7**, **V/12** und **V/15** führten, einen positiven Effekt auf die Potenz der Inhibitoren hatten. Im Gegensatz dazu scheint eine planare Anordnung der Aromaten Voraussetzung für eine gute inhibitorische Aktivität an CYP11B2 zu sein, damit zeigten die *ortho*-substituierten Verbindungen zusätzlich eine sehr gute Selektivität.

Zusätzlich hervorzuheben ist, dass H-Brücken-Donatoren in *para*-Position (OH, **V/11**; NH_2, **V/17**) einen positiven Effekt auf die Aktivität der Inhibitoren haben. Alle *para*-Substituenten ohne diese Eigenschaft erniedrigten die CYP11B1-Hemmung im Vergleich zu **IV/33**.

Verschiedene Annellierungen von Benzolkernen, sowohl an den Phenylring von **IV/33** als auch an verschiedene Heterozyklen, konnte die Hypothese bestätigen, dass *ortho*-Substitution sehr vorteilhaft für eine hohe Aktivität und auch Selektivität der Inhibitoren ist. Dieses Substitutionsmuster beinhaltet auch die beste Verbindung dieser Studie, das 1-Naphthalin **V/23**. Dieser Inhibitor zeigt eine dem klinisch genutzten Metyrapon vergleichbare Aktivität, allerdings eine deutlich höhere Selektivität. Er ist somit der selektivste CYP11B1-Inhibitor, der bislang beschrieben wurde und damit ein aussichtsreicher Kandidat für weitere Entwicklungen. Dieser Wirkstoff könnte nicht nur zur Therapie des Cushing-Syndroms eingesetzt werden, sondern wäre auch eine mögliche Therapieoption beim Metabolischen Syndrom, da hier erhöhte Cortisolplasmaspiegel ebenfalls beobachtet werden. Dazu lieferte eine kürzlich veröffentlichte Studie mit dem 2*S*-4*R*-Enantiomer von Ketokonazol (DIO-902) an Patienten mit Typ2-Diabetes und weiteren Krankheitsbildern des metabolischen Syndromes vielversprechende Ergebnisse.[32] Es konnte gezeigt werden, dass die Verabreichung von DIO-902 zu erniedrigten HbA1c-, Cholesterin- und Blutdruckwerten führte und gleichzeitig konnte eine Gewichtsreduktion bei den Patienten beobachtet werden.

So lässt sich ingesamt sagen, dass mit den CYP11B1-Inhibitoren, die in dieser Arbeit entwickelt wurden, eine höchst interessante Strukturklasse dargestellt wurde. Nach einer Weiterentwicklung bezüglich ihrer pharmakologischen Eigenschaften sollten mit diesen Verbindungen erste hochwirksame Wirkstoffe zur Therapie das Cushing-Syndroms sowie Krankheiten, die mit erhöhten Cortisolspiegeln einhergehen, zur Verfügung stehen.

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