Heme-Iron Complexing Biphenyl and Naphthalene Derivatives as CYP17 Inhibitors for the Treatment of Prostate Cancer: Design, Synthesis and Evaluation

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

von
M. Sci. Mariano Aurelio Ernesto Pinto-Bazurco Mendieta

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Dekan:	Prof. Dr. U. Müller
Berichterstatter:	Prof. Dr. R. W. Hartmann

‘Obstacles are those frightful things you see when you take your eyes off your goal’

Henry Ford

Diese Arbeit entstand unter der Anleitung von Prof. Dr. Rolf W. Hartmann in der Fachrichtung 8.2 Pharmazeutische und Medizinische Chemie der Naturwissenschaftlich- Technischen Fakultät III der Universität des Saarlandes von September 2004 bis Dezember 2008.

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to those affected in any way by cancer

Articles presented in this work

The present work is mainly published in three articles:

I. 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-Vieira, Dirk Schmidt, Thomas Lauterbach, and Rolf W. Hartmann

Arch. Pharm. Chem. Life Sci. **2008**, *341*, 597–609

II. Synthesis, biological evaluation and molecular modelling 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, Ursula Müller-Vieira, Dirk Schmidt, Klaus Hansen, and Rolf W. Hartmann

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

III. Synthesis, Biological Evaluation, and Molecular Modeling of Abiraterone Analogues: Novel CYP17 Inhibitors for the Treatment of Prostate Cancer

Mariano A. E. Pinto-Bazurco Mendieta, Matthias Negri, Carsten Jagusch, Ursula Müller-Vieira, Thomas Lauterbach, and Rolf W. Hartmann

J. Med. Chem. **2008***, 51*, 5009–5010

as well as in two other co-authored articles:

A. Synthesis, Biological Evaluation and Molecular Modelling Studies of Methyleneimidazole Substituted Biaryls as Inhibitors of Human 17α-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-Hoffman, Mariano A. E. Pinto-Bazurco Mendieta, Barbara Rodenwaldt, Ursula Müller-Vieira, Dirk Schmidt, Thomas Lauterbach, Maurizio Recanatini, Andrea Cavalli, and Rolf W. Hartmann.

Bioorg. Med. Chem. **2008**, *16*, 1992–2010

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

Ulrike Hille, Qingzhong Hu, Mariano A. E. Pinto-Bazurco Mendieta, Marc Bartels, Carsten A. Vock, Thomas Lauterbach, and Rolf W. Hartmann.

Comptes Rendus Chimie, accepted

and one more manuscript in draft soon to be submitted to international scientific journals.

Besides the above mentioned articles, the data and results regarding the preparation and evaluation of another considerable amount of substances designed and synthesized by the author throughout this research period had to be left out of this work due to secrecy reasons, and are therefore not shown or discussed herein. The mentioned data and results are currently being submitted for one or two separate disclosures of invention, for the treatment of prostate cancer with different therapeutical approaches, and are also to be published further on.

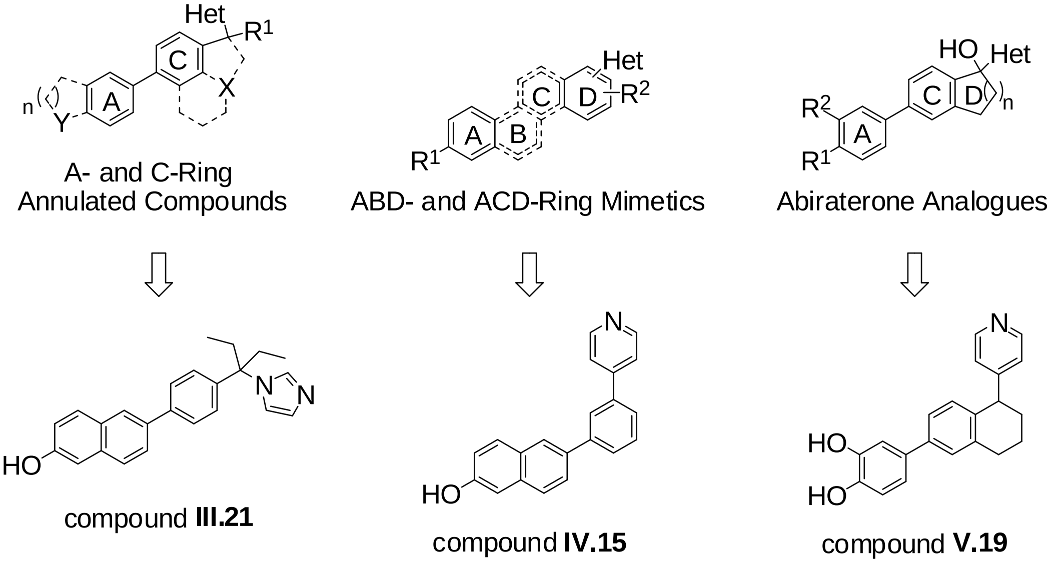
Contribution Report

In the following, the author wishes to clarify his contributions to the articles presented and discussed within this work.

Besides significantly contributing to the inhibitor design concept of the compound classes enclosed in this work, he also significantly contributed to the interpretation of the results and wrote the respective manuscripts. Furthermore, he planned, synthesized and also characterized all the corresponding compounds, except for compounds further on referred to as **III.4, III.5, III.19**-**III.21** and **IV.17**, synthesized by Ulrike E. Hille, to whom he is sincerely thankful for her kind contribution.

Abstract

Insufficient efficacy and unsafe treatment options are the current state of the art of prostate cancer therapies. This motivated us to develop antiandrogenic drugs with reduced side effects, aiming at effective tumor growth prevention and regression for prostatic adenocarcinomas. The key enzyme in androgen synthesis CYP17 is to date the most promising target for an overall, mild treatment of prostate carcinomas. Thus, it was our aim to develop nonsteroidal CYP17 inhibitors. In the present work, the synthesis, biological evaluation and molecular modeling studies of 69 compounds divided in three main classes are presented. These were published in three different international scientific magazines. A further manuscript is already prepared. Other related compounds, not essential for this work, are already published or to be published soon in four other co-authored works. The compounds discussed in this work belong to the following classes:

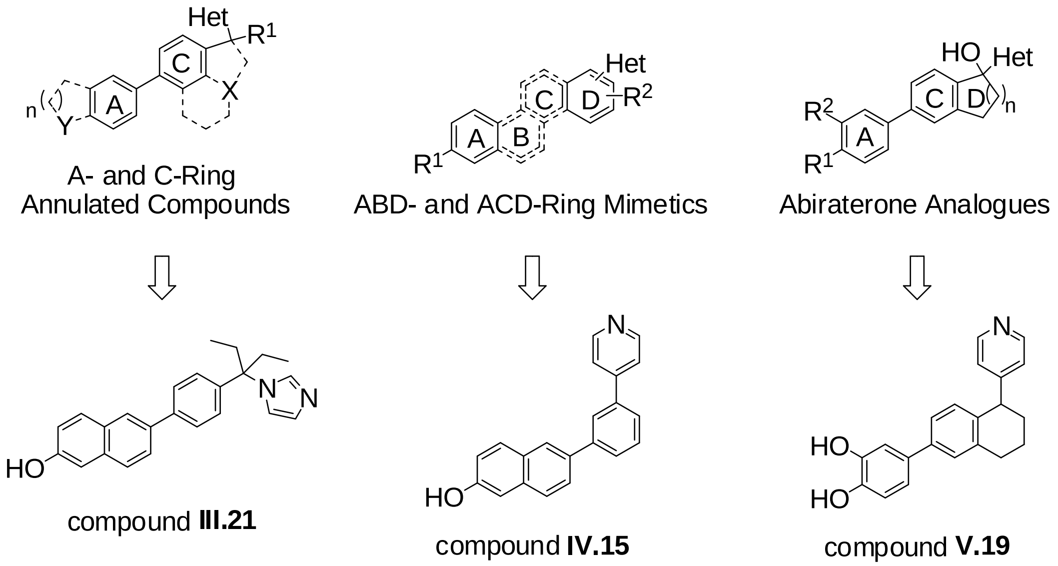


Presented classes and their lead representants.

III.21 and **IV.15** were moderate active and selective, while **V.19** exhibited 144 nM IC_{50}, good CYP3A4 and excellent CYP11B2 and CYP11B1 selectivity. These biological activities were discussed, and structural changes, for activity and selectivity improvement are proposed. Enantiomeric separation and further *in vitro* and *in vivo* tests are still undergoing. In case these are successful, clinical trials will be planned.

Zusammenfassung

Ungenügend wirksame und risikoreiche Behandlungen sind der heutige Stand der Prostatakrebstherapien. Dies motivierte uns zur Entwicklung antiandrogener nebenwirkungsarmer Wirkstoffe mit dem Ziel eine effektive Tumorwachstumprävention und -regression prostatischer Adenokarzinome zu erreichen. Das Schlüsselenzym der Androgensynthese CYP17 ist bis dato das vielversprechendste Target für eine Behandlung des Prostatakarzinoms. Dementsprechend war es unser Ziel nicht-steroidale CYP17 Inhibitoren zu entwickeln. In der vorliegenden Arbeit wurde die Synthese, biologische Evaluierung und Molecular Modeling Studien von 69 Verbindungen in drei Substanzklassen vorgestellt. Diese wurden in drei verschiedenen internationalen Zeitschriften publiziert. Ein weiteres Manuskript ist fertiggestellt. Andere verwandte, für diese Arbeit nicht essentielle Verbindungen, sind in vier weiteren Publikationen niedergelegt. Die vorgestellten Verbindungen gehören folgenden Stoffklassen an:



Vorgestellte Verbindungen und entsprechende Lead-Vertreter.

III.21 und **IV.15** waren moderat aktiv und selektiv, während **V.19** mit 144 nM IC_{50} gut aktiv war, gute CYP3A4 und exzellente CYP11B2 und CYP11B1 Selektivität zeigte. Die biologischen Aktivitäten wurden diskutiert, und strukturellen Änderungen zur Verbesserung der Aktivität und Selektivität vorgeschlagen. Enantiomerentrennung und weitere *in vitro* und *in vivo* Tests werden gegenwärtig durchgeführt. Für den Fall, dass diese erfolgreich sind, werden klinische Studien geplant.

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List of Abbreviations

µ, micro

AD, anno Domini

AS, androgen suppression

Ar, aromatic heterocycle

Arg, arginine

Asn, asparagine

ATR, attenuated total reflection

BC, before Christ

Bu, butyl

CAB, combined androgen blockade

CH_2Cl_2, dichloromethane

CYP, cytochrome P450

CYP11B1, cytochrome P450 steroid-11-*β*-hydroxylase

CYP11B2, cytochrome P450 18-hydroxylase

CYP17, cytochrome P450 17Rhydroxylase-17,20-lyase

DCM, dichloromethane

DNA, desoxyribonucleic acid

E. coli, Escherichia coli

ESI, electrospray ionization

et al, et alii

Et, ethyl

g, gram

GA, genetic algorithm

Gly, glycine

GnRH, gonadotropin-releasing hormone

h, hour

H_2O, water

H-bond, hydrogen-bond

Het, heterocycle

His, histidine

HPLC, high performance liquid chromatography

HRPC, hormone-refractory prostate cancer

i, iso

Ile, isoleucine

IR, infrared

L, liter

LC-MS/MS, liquid chromatography - tandem mass spectrometry

Lys, lysine

m, milli

M, molar

Me, methyl

MHz, megahertz

min, minutes

mp, melting point

n, nano

Na_2CO_3, sodium carbonate

Na_2SO_4, sodium sulfate

$NaBH_4$, sodium borohydride

NH_4Cl, ammonium chloride

NMP, N-methyl pyrrolidine

NMR, nuclear magnetic resonance

OAc, acetate

OH, hydroxyl

PC, prostate cancer

$Pd(PPh_3)_4$, tetrakis-(triphenylphosphine) palladium(0)

PDB ID, protein data bank identification

PDB, Protein Data Bank

Phe, phenylalanine

RNA, ribonucleic acid

rt, room temperature

Si, silica

t, tertiary

THF, tetrahydrofuran

Thr, threonine

TMS, trimethylsilane

USA, United States of America

UV, ultraviolet

I. Introduction

from www.astrologycom.com/vitalism.html

I.1. Cancer

I.1.a. Quid est cancer?

The first records of cancer are attributable to Hippocrates (460-370 BC), founder of medicine, who first used the Greek terms καρκίνος (‘carcinos,’ *gr.* crayfish) and καρκίνομα (‘carcinoma’) to refer to and describe in detail chronic ulcers or growths that seemed to be malignant tumors, most likely because the finger-like spreading projections from a cancer called to mind the shape of a crab. Benign tumors were called ονκος (‘oncos,’ the root of the modern word oncology, the study of tumors), Greek for swelling or bulk. More than 400 years later, Celsus (28 BC - 50 AC) referred to ‘carcinos’ with the Latin translation ‘cancer’ to refer to malignant, invasive tumors, and introduced the Greek work ‘carcinoma’ as such in Latin for superficial malignant and premalignant tumors. Galen (130-200 AC) also contributed to the early history of oncology with many scientific observations, and used on his part the Greek term ‘oncos’ to refer also to a malignant-looking tumor.[1]

Cancer is a term used for a group of more than 100 diseases in which abnormal cells begin to grow out of control and order and are able to invade other tissues. It is estimated to be the actual most common cause of death in the US, succeeded by coronary disease, cerebrovascular diseases, respiratory diseases and unintentional injuries (accidents).[2] Normal body cells grow, divide, and die in a controlled way as they are needed to keep the body healthy. However, the genetic material (DNA) of a single human cell becomes damaged or changed by environmental factors, like chemicals, viruses, tobacco smoke or too much sunlight about 10,000 times every day, producing mutations that affect normal cell growth and division. Most of the time when DNA becomes damaged, either the cell commits suicide (apoptosis) or is able to detect and repair the DNA by itself. In cancer cells, the damaged DNA is not repaired and new cells form when the body does not need them. These new cells are created by the division (mitosis) of the cell nucleus. In this way, the damaged DNA, which is located in the cell nucleus, gets duplicated and the newly formed cells again carry the same damaged DNA. People can also inherit damaged DNA, which accounts for inherited cancers.

These extra cells may form a mass of tissue called a growth or a tumor. Some cancers though, like leukemia, do not form tumors, but instead, the cancer cells involve the blood and blood-forming organs, and circulate through other tissues where they grow. In the same manner, not all tumors are cancerous. Cancer cells can spread to other parts of the body through the bloodstream and lymph systems where they begin to grow and replace normal tissue. Such spreading process is called *metastasis*. Noncancerous (*benign*) tumors do not metastasize and are very rarely life-threatening.

Cancers can begin in many different parts of the body and are mostly named after the organ or type of cell in which they start. As an example, prostate cancer is the kind of cancer which begins in the prostate. Even when cancer has spread to a different part of the body it is still named after the place in the body where it started, e.g., prostate cancer that has spread to the bone is called metastatic prostate cancer, not bone cancer. This is very important, since different types of cancer can act very differently. For example, prostate cancer and lung cancer are very different diseases. They grow at different rates and respond to different treatments. That is why people with cancer need treatment that is aimed at their particular kind of cancer.

Cancer types can be grouped into broader categories. The main categories of cancer include:

- Carcinoma: cancer that begins in the skin or in tissues that line or cover internal organs.
- Sarcoma: cancer that begins in bone, cartilage, fat, muscle, blood vessels, or other connective or supportive tissue.
- Leukemia: cancer that starts in blood-forming tissue such as the bone marrow and causes large numbers of abnormal blood cells to be produced and enter the blood.
- Lymphoma and myeloma: cancers that begin in the cells of the immune system.
- Central nervous system cancers: cancers that begin in the tissues of the brain and spinal cord.

Although cancer is not contagious, its occurrence is very high. Half of all men and one-third of all women in the US will develop cancer during their lifetimes. The risk of developing most types of cancer can be reduced by changes in a person's lifestyle, for example, by quitting smoking, limiting time in the sun, being physically active, and eating a better diet.[3] The sooner a cancer is found and treated, the better the chances are for living for many years.

I.1.b. Risk Factors and Prevention

Anything that may increase a person’s chance of developing a disease is called a risk factor; anything that may decrease a person’s chance of developing a disease is called a protective factor. A risk factor shows a higher incidence in a test group to develop cancer, but it does not mean that it will increase your chance to get cancer. Most people who have risk factors never develop cancer while also some people are more sensitive than others to the known factors.

The most important risk factor for cancer is growing older. Most cancers occur in people over the age of 65, but still, even children can get cancer, too. While growing older is not preventable, the most important, preventable cause of cancer death is tobacco use. Each year, more than 180,000 USA citizens die from cancer that is related to tobacco use. Smokers are more likely than nonsmokers to develop cancer of the lung, larynx, mouth, esophagus, bladder, kidney, throat, stomach, pancreas, cervix and acute myeloid leukemia.

The most important environmental cause of cancer is ultraviolet (UV) radiation. It causes early aging of the skin and skin damage that can lead to skin cancer. It is best to avoid the midday sun, use sunscreen with a sun protection factor (SPF) of at least 15 and stay away from sunlamps and tanning booths. Ionizing radiation can also cause cell damage that leads to cancer. This kind of radiation comes from rays that enter the Earth's atmosphere from outer space, radioactive fallout, radon gas (a gas formed in soil and rocks), x-rays, and other sources. Wide bodies of water like lakes charge the air negatively, counteracting the cancerous positively-charged air.

Also ingestion or exposure to certain substances may increase the risk of cancer. Many studies have shown that exposure to asbestos, benzene, benzidine, cadmium, nickel, vinyl chloride and many other chemicals may cause cancer. Having more than two drinks of alcohol each day for many years, too, may increase the chance of developing cancers of the mouth, throat, esophagus, larynx, liver and breast. Physicians advise people to drink no more than one drink per day for women and no more than two drinks per day for men.

Other risk factors are of biological nature. Being infected with certain viruses or bacteria such as: human papilloma viruses (HPVs), hepatitis B and C viruses, human T-cell leukemia/lymphoma virus (HTLV-1), human immunodeficiency virus (HIV), Epstein-Barr virus (EBV), human herpes virus 8 (HHV8) and helicobacter pylori may increase the risk of developing cancer. Also, certain hormones are an important risk factor for cancer. For example high blood levels of testosterone may increase the risk of prostate cancer, while otherwise low levels are a marker for developing heart diseases and metabolic syndrome diabetes. The family history of cancer may also be a significant risk factor, since some gene changes that increase the risk of cancer are passed from parent to child. Certain types of cancer like melanoma and cancers of the breast, ovary, prostate, and colon occur more often in some families than in the rest of the population. However, environmental factors may also be involved and most of the time, multiple cases of cancer in a family are just a matter of chance.

Furthermore, dietary habits and not enough physical activity or being overweight may increase the risk of several types of cancer. Studies suggest, that people, whose diet is high in fat have an increased risk of cancers of the colon, uterus and prostate. Lack of physical activity and being overweight are risk factors for cancers of the breast, colon, esophagus, kidney and uterus. A healthy diet includes a high variety of food high in fiber, vitamins and minerals, and less food high in fat. In addition, moderate physical activity (like brisk walking) for at least 30 minutes on 5 or more days each week is recommended to help control your weight and reduce body fat.

I.1.c. Classification, Treatment and Prognosis

The process of diagnosing a cancerous clinical condition has two parts: grading (determining the tumor grade), to establish the pathology (classify the cytology), and staging, to determine the advance and implications of the cancer. The classification of a cancer within these two main criteria helps develop an individual treatment plan for the patient and predict the patient's prognosis. Generally, a lower degree of each indicates a better prognosis: the likely outcome or course of a disease as well as the chance of recovery or recurrence.

Tumor grade is a system used to classify cancer cells in terms of how abnormal they look under a microscope and how quickly the tumor is likely to grow and spread. It ranges G1 to G4 from well-differentiated (low grade) to undifferentiated (high grade) tumors and GX, if the grade cannot be assessed.[4]

Many factors are considered when determining the tumor grade, as the specific factors vary with each type of cancer. The two main factors are histological and nuclear grade. Histological grade, also called differentiation, refers to how much the tumor cells resemble normal cells of the same tissue type. Nuclear grade refers to the size and shape of the nucleus in tumor cells and the percentage of tumor cells that are dividing.

Grading systems are also different for each type of cancer. For example, to describe the degree of differentiation of prostate cancer cells the Gleason system is used. The Gleason system uses scores ranging from Grade 2 (well-differentiated) to Grade 10 (undifferentiated). Other grading systems include the Bloom-Richardson system for breast cancer and the Fuhrman system for kidney cancer.

On the other hand, cancer stage refers to the extent or severity of the cancer, based on factors such as the location of the primary tumor, tumor size, number of tumors, and lymph node involvement (spread of cancer into lymph nodes), but also on the tumor grade. For most cancers, the stage is rated 0 (pre-cancerous abnormal carcinoma *in situ*, the primary tumor is present only where it began) to IV (the primary tumor has spread to other organs).

These stages are usually based upon the TNM system. It considers the extent of the tumor (T), the extent of spread to the lymph nodes (N), and the presence of metastasis (M). A degree is added to each letter to indicate the size or expansion of the tumor and the extent of spread. The degrees for classifying the primary tumor are: TX (primary tumor cannot be evaluated), T0 (no evidence of primary tumor), Tis (carcinoma *in situ*, which means it has not spread to neighboring tissue) and T1 to T4 to define a growing size and/or extent of the primary tumor. For the regional lymph nodes, the degrees are analogous: NX, N0 and N1 to N3 (number and/or extent of spread). As for the distant metastasis, MX means it cannot be evaluated, and M0 and M1 describe respectively absence or presence of such. To illustrate this, prostate cancer T2 N0 M0 means that the tumor has begun to spread outside the prostate, but has not spread to the lymph nodes or any other part of the body. Nevertheless, it is to observe, that criteria for stages differ for different types of cancer. For example, prostate cancer T3 N0 M0 is stage III; however, colon cancer T3 N0 M0 is a stage II cancer.

The tests used for staging depend on the type of cancer. They include physical exams by looking, feeling, and listening for anything unusual as well as imaging studies (x-rays, computed tomography (CT) scans, magnetic resonance imaging (MRI) scans, and positron emission tomography (PET) scans), which produce pictures of areas inside the body, both useful in showing the location of the cancer, the size of the tumor, and whether the cancer has spread to the lymph nodes and/or to other organs. Laboratory tests of blood, urine, other fluids and tissues taken from the body can provide diverse information about the cancer. A biopsy (the removal of cells or tissues for examination under a microscope) is usually performed to provide pathology reports, which may include information about the size of the tumor, the growth of the tumor into other tissues and organs, the type of cancer cells, and the grade of the tumor. Cytology reports also describe findings from the examination of cells in body fluids. Surgical reports describe the size and appearance of the tumor and often include observations about lymph nodes and nearby organs found during surgery.

Surgery is the oldest form of cancer treatment and, depending on the kind and stage of the cancer, it may vary from a small procedure up to much bigger operations. Besides diagnostic and staging surgery, there are several other types of surgery like prophylactic (preventive), curative (when it is likely to remove the entire tumor), debulking or cytoreductive (to remove a part of the tumor when not all may be removed), supportive (supporting other treatments) and restorative (a reconstructive surgery to restore biological functions or the way a person looks after another surgery). Surgery is usually the first treatment to be done, accompanied by radiation or chemotherapy.

Radiation therapy rests on the higher radiosensitivity of quickly dividing cells like cancerous cells. By interfering mitosis (cell division) through an ionizing radiation, mainly cancerous cells are affected. The damage made to normal cells is what causes side effects. It is usually used for curing or shrinking early stage cancer, stopping cancer from recurring in another area, but also for treating symptoms of advanced cancers. Because of the damage caused to the normal cells, only in some cases a second course of radiation therapy can be given. The most common types of radiation are: high-energy photons (most common, radiation comes from cobalt, cesium or a linear acceleration machine or 'linac'), electron beams (for tumors close to a body surface), protons (more effective and with fewer side effects) and neutrons (used for cancers of the head, neck and prostate, has severe long-term side effects). Minor side effects include fatigue (most common, but the exact cause is unknown), skin lesions and others related to the damage produced to the treated organs, while the most important side effect is the chance to develop second cancers, which relies on changes in the DNA caused by the radiation itself. These may appear after 5 years, up to more than 15 years after treatment.

The word chemotherapy was once used to mean any treatment with any medicine. Mustard gas was the first chemotherapeutic to be used for the treatment of cancer during the 1940s. The classical administration form of chemotherapeutics is intra venous (i. v.), though sometimes other options, like intra muscular (i. m.) and per oral (p. o.), are also possible. The appropriate chemotherapeutics, from over 100 systematically used, must be chosen depending on cancer type, grade and stage. They can be used to cure the cancer, control it from growing and spreading, and to relieve symptoms and improve the quality of life (palliative) when a fully-recovery is not possible. This treatment can be used alone or in combination with others, before (neoadjuvant) or after (adjuvant) the main treatment. Classical chemotherapeutics may be divided into cytostatic – growth interfering, and cytotoxic – cell-death inducing.

Chemotherapeutics may also be classified according to their mode of action in DNA interfering (alkylating agents, antimetabolites, antibiotics, topoisomerase inhibitors), microtubules interfering (vinca alkaloids, taxanes) and hormonal agents. Alkylating agents (cisplatin, carboplatin, cyclophosphamide, chlorambucil) attach alkyl groups to DNA strings leading to cell death. Antimetabolites (mercaptopurine, thioguanine, 5-fluorouracil (5FU), methotrexate) replace DNA and RNA bases (both purinic and pyrimidinic) and are also cytotoxic. Anthracyclines (doxorubicine, epirubicine) and mitoxantrone (an anthracenedione, similar in structure to anthracyclines) are antibiotics, which intercalate between bases in a DNA string. Topoisomerase inhibitors like etoposide, teniposide, irinotecan and topotecan interfere with the folding and unfolding of DNA strings, thus interfering with transcription (coding) and replication of DNA. Vinca alkaloids (vincristine, vinblastine) and taxanes (paclitaxel, docetaxel) prevent microtubule (a component of the cytoskeleton) function and are cytostatic. Hormonal agents are hormones, derivatives or other small molecules which interfere with the hormonal household, responsible for tumor growth in some cancers like breast (estrogen receptor modulators: tamoxifen; aromatase inhibitors: letrozole, fadrozole) and prostate cancer (antiandrogens, GnRH analogs, 5α-reductase inhibitors, CYP17 inhibitors), and for treating side effects (dexamethasone). Antisense therapeutics for neoplastic diseases are still in research. Their mode of action relies on the interference of a synthetic strand of DNA or RNA with the messenger RNA (mRNA), produced by a gene which is to be responsible for the abnormal function of cells, thus turning the gene off.

These medicines are usually given in cycles to reduce the harm to healthy cells and allow the drugs to kill more cancer cells. The exact course and duration of the therapy depends on each clinical condition. During therapy, diverse side effects, which vary from patient to patient, may be observed. This relies on the fact, that chemotherapeutics usually target in a greater degree all quickly dividing cells in the body such as cells that grow hair (leads to hair loss), cells in your bone marrow (may cause tiredness, easy bruising and a higher risk of infection), cells of the skin and mouth (dryness and sores in mouth and skin), and cells in your stomach and intestines (vomit, diarrhea). They may also cause loss of libido.

Immunotherapy relies on the immune system for lymphocytes, antigen-presenting cells (monocytes, macrophages, dendritic cells) and antibodies to kill cancerous cells, either by modifying the patient’s own immune system (cancer vaccines, nonspecific immunotherapies and adjuvants), by the introduction of foreign antibodies (monoclonal antibodies), and by using antibodies to deliver other drugs to a cancerous cell, but also on other, nonimmunorelated mechanisms. Cancer vaccines are classified into tumor cell (made from killed tumor cells), antigen (by using only one or a few antigens), dendritic cell (with the most cancer-specific antigens), anti-idiotype (for the specific idiotype for the precise kind of cancer) and DNA vaccines (to instruct cells to create antigens) and are all still under research. Nonspecific immunotherapies include the use of cytokines (interleukins, interferons) and many other adjuvant therapeutics (Bacille Calmette-Guérin (BCG), detox, qs-21, keyhole limpet hemocyanin (KLH)) to regulate and improve the patient’s immune system. Classical (‘naked’) monoclonal antibodies target specific cancerous cells and induce cell-death (rituximab, alemtuzumab). Other naked monoclonal antibodies attach themselves to cancerous cells, disrupting specific growth processes like mitosis (trastuzumab, cetuximab), angiogenesis (development of new cancer supporting blood vessels, bevacizumab) and other processes. Conjugated monoclonal antibodies deliver other drugs (currently under study), toxins (under study) or radioactive substances (ibritumomab, tositumomab) to cancerous cells.

Treatment options and prognosis depend not only on the stage of the cancer, but also on the patient’s health and most importantly on whether the cancer has just been diagnosed or has recurred (come back).

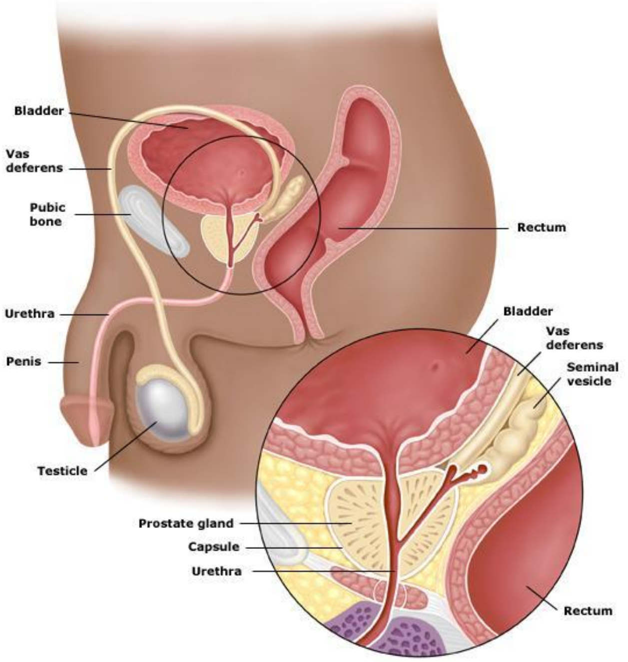
I.2. The Prostate

I.2.a. Biological Function

The word prostate derives from the Greek word προστάτης (‘prostates’) and literally means ‘one standing before.’ It is a walnut-sized tubuloalveolar (its secretory portions end in tubular and alveolar configurations) exocrine (secrete their products into ducts) gland of the male mammalian reproductive system. The prostate starts to develop before birth and continues to grow until a man reaches adulthood. Its secretion is slightly alkaline (pH 7.29) to neutralize the acidity of the vaginal tract (prolonging the lifespan of sperm) and constitutes 10-30% of the volume of the seminal fluid that, along with spermatozoa, constitutes semen. The rest of the seminal fluid is produced by the two seminal vesicles.[5]

The prostate (Figure 1) is located between the lower part of the rectum and the pubic bone. It is sheathed in the muscles of the pelvic floor, which contract during the ejaculatory process, and surrounds the urethra just below the urinary bladder. Within the prostate, the urethra coming from the bladder is called prostatic urethra and merges with the two ejaculatory ducts. The seminal vesicles are located behind the bladder. The excretory ducts of the seminal glands open into the vas deferens as it enters the prostate gland.

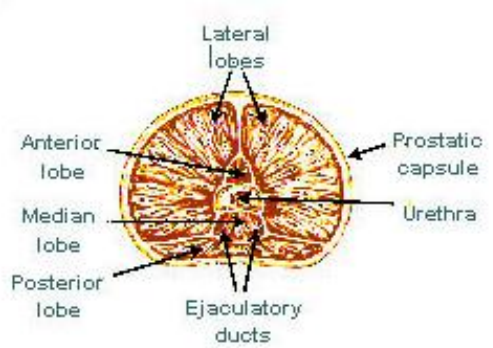
The prostate can be sectioned into lobes and zones, the lobe classification (Figure 2) being more often used in anatomy, while the zones classification (Figure 3) is useful for pathology studies.



from http://www.utdol.com/patients/content/topic.do

Figure 1. Human male urogenital system including the penis, testicles, seminal vesicles and prostate.

The anterior lobe is used to describe the anterior portion of the gland lying in front of the urethra. It is devoid of glandular tissue being formed completely of fibromuscular tissue. The median lobe is a cone-shaped portion of the gland situated between the two ejaculatory ducts and the urethra. The lateral lobes (right and left lobes) form the main mass of the gland. They are separated by the prostatic urethra. The posterior lobe is used by some to describe the postero-medial part of the lateral lobes that can be palpated through the rectum during digital rectal examination (DRE, see chapter I.3.b).



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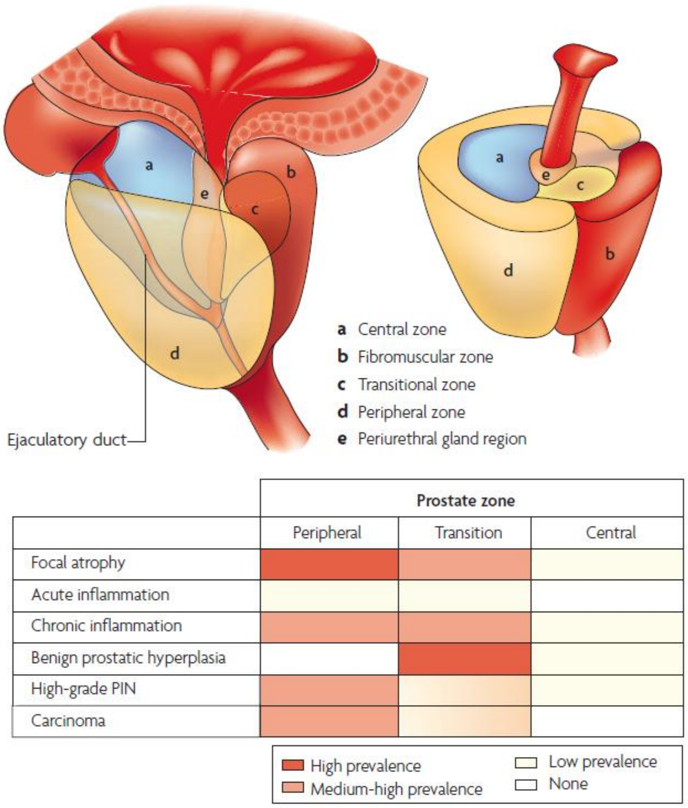


	Prostate zone		
	Peripheral	Transition	Central
Focal atrophy	🟥	🟧	🟨
Acute inflammation	🟨	🟨	⬜
Chronic inflammation	🟧	🟧	🟨
Benign prostatic hyperplasia	⬜	🟥	🟨
High-grade PIN	🟧	🟨	🟨
Carcinoma	🟧	🟨	⬜

🟥 High prevalence
🟧 Medium-high prevalence
🟨 Low prevalence
⬜ None

Figure 2 (up). Prostate horizontal sectional view. Division in 5 lobes: anterior, median and posterior lobes and two (left and right) lateral lobes.

Figure 3 (right). Prostate division into 4 zones: central, fibromuscular, transitional and peripheral zones, and periurethral gland region. Prevalence of prostatic disorders by zone.

from http://demarzolab.pathology.jhmi.edu/Reprints/nrc2090_published.pdf

The prostate gland has four distinct glandular regions, two of which arise from different segments of the prostatic urethra:

The peripheral zone (PZ) composes up to 70% of the normal prostate gland in young men. It is the sub-capsular portion of the posterior aspect of the prostate gland which surrounds the distal urethra. It is from this portion of the gland that more than 70% of prostatic cancers originate.

The central zone (CZ) constitutes approximately 25% of the normal prostate gland. This zone surrounds the ejaculatory ducts. Central zone tumors account for more than 25% of all prostate cancers.

The transitional zone (TZ) is responsible for 5% of the prostate volume. This zone is very rarely associated with carcinoma. It consists of two paraurethral (next to the urethra) lobules anterior to the peripheral zone. The transition zone surrounds the proximal urethra, and is the region of the prostate gland which grows throughout life and is responsible for the disease of benign prostatic enlargement or hyperplasia (BHP).

The anterior fibro-muscular zone (or stroma) accounts for approximately 5% of the prostatic weight. This zone is usually devoid of glandular components and composed only, as its name suggests, of muscle and fibrous tissue.

The last anatomically discrete area within the glandular prostate is the periurethral (surrounding the urethra) gland region, which represents less than 1% of the total volume of the glandular prostate. Its ductal network represents a more proximal extension of the networks of the peripheral and transition zone areas.

I.2.b. Prostatic Disorders

The most common prostatic disorders are of inflammatory or neoplastic (development of new tissues) nature: prostatitis, benign prostate hyperplasia (BPH) and prostate cancer.[6]

Prostatitis is literally the inflammation of the prostate gland. In modern medicine, it is a commonly used term for several different complications in the urogenital system between the pelvic bone and the rectum, which are not always attributable to inflammation of the prostate. Symptoms of a clinically relevant prostatitis are pain and difficulty while urinating (alguria), frequent urination (pollakisuria), complications of the urinary flow, and pain during and especially after ejaculation. No specific markers exist for the diagnosis of prostatitis, and since the symptoms are similar to those of other prostate disorders, the same diagnostic tests are performed. There are different forms of prostatitis, each with different causes and outcomes.

Acute prostatitis can lead to urinary obstruction, and in such way chills and fever. It is usually of bacterial nature, commonly from *E. coli* bacteria, which are found in the urine. Both the leukocyte count and the prostate specific antigen (PSA, see chapter I.3.b) levels are high. It is successfully treated with antibiotics such as fluoroquinolone and tetracyclines. Chronic bacterial prostatitis is the milder, more difficult to treat form of acute bacterial prostatitis, but the treatment remains the same.

Chronic nonbacterial prostatitis or male chronic pelvic pain syndrome, which comprises about 95% of all prostatitis diagnoses, can have many different causes: from an autoimmunological failing, over neurogenical inflammation up to myofascial pain syndrome (MPS, a painful musculoskeletal condition). There are two kinds of chronic pelvic pain syndrome: inflammatory and noninflammatory. Treatments for either clinical condition include alpha blockers, phytotherapy, physical therapy, psychotherapy, antihistamines, anxiolytics, nerve modulators and more. More recently, a combination of trigger point and psychological therapy has proved to be effective as well.

Benign prostatic hyperplasia (BPH) is the noncancerous enlargement of the prostate, so that it obstructs the urethra. It is one of the most common pathologies in elderly men, accounting for 25-40% of men over 50 years old being diagnosed with this disease. Symptoms include pollakisuria, or taking some time to start urinating (hesitancy). Common treatment for BPH includes 'watchful waiting' until the disease develops into a more serious clinical condition, medication, a minimally invasive procedure or, in extreme cases, surgery that removes the prostate (prostatectomy).

For the treatment of BHP, medication has to be considered depending on the goals of the treatment. Alpha blockers provide symptomatic relief of BPH symptoms. Available drugs include doxazosin, terazosin, alfuzosin and tamsulosin. 5α-reductase inhibitors (finasteride, dutasteride) are another treatment option for stopping the prostate from growing.

Minimally invasive procedures include transurethral (through the urethra) needle ablation (removal of tissue) of the prostate (TUNA) and transurethral microwave thermotherapy (TUMT). These outpatient procedures may be followed by the insertion of a temporary prostatic stent, to allow normal voluntary urination, without exacerbating irritative symptoms.

The surgery which is most often performed in such cases is called transurethral resection of the prostate (TURP or TUR). In TURP, an instrument is inserted through the urethra to remove prostate tissue that is pressing against the upper part of the urethra and restricting the flow of urine. Older men often have corpora amylacea (amyloids), dense accumulations of calcified proteinaceous material, in the ducts of their prostate. The corpora amylacea may obstruct the lumens (interior space) of the prostatic ducts, and may underlie some cases of BPH. Also urinary frequency due to bladder spasm, common in elderly men, may be confused with prostatic hyperplasia.

It is believed that prostate cancer begins with a pre-cancerous condition called prostatic intraepithelial neoplasia (PIN). PIN begins to appear in some men in their 20s and almost half of all men have PIN by the time they reach 50. Even though PIN cells look abnormal under the microscope, they are not invasive, but a high-grade PIN increases the probability by 20%, that the patient already has prostate cancer. Another possible finding during a biopsy is atypical small acinar proliferation (ASAP), also called atypia. Atypia cells look cancerous, but there are too few of them to be certain. Nevertheless, the chance of already having prostate cancer is of 40-50%. A third finding may be proliferative inflammatory atrophy (PIA). The cells look abnormal under the microscope, and they may turn into prostate cancer directly or by first changing into high-grade PIN.

Prostate cancer is one of the most common cancers affecting older men in developed countries. Although it is very often diagnosed at an early stage, it is a significant cause of death for elderly men. It is like other cancers, a complex disease with many risk factors: race, age, genetics, and lifestyle habits can all contribute to its development.

The most common site of origin of prostate cancer is in the peripheral zone (the main glandular zone of the prostate), thus prostatic adenocarcinoma (adeno – *lat.* gland), the most common prostate cancer, is not always easy to detect through a DRE (see chapter I.3.b).

Small cell carcinoma is made up of small round cells, and typically forms at nerve cells. Small cell carcinoma is very aggressive in nature and, as it does not lead to an increase in PSA, it can be somewhat harder to detect than adenocarcinoma; this usually means that it has reached an advanced form upon detection.

Squamous cell carcinoma is a nonglandular cancer. Similar to small cell carcinoma, there is no increase in PSA when it is present and is also very aggressive in nature.

Adenoid cystic/basal cell carcinoma (ACBCC) is a rare neoplasm in the prostate. It is the most common kind of skin cancer, and in the prostate it is a cancer of the prostatic capsule. Recent research reveals that it may be of aggressive nature since it may easily develop extraprostatic (outside the prostate) extension and metastasis.

There are other, more rare, forms of prostate cancer. These include sarcomas and transitional cell carcinoma; the latter rarely develops in the prostate but derives from primary tumors present in the bladder or urethra.

I.3. Prostate Cancer

I.3.a. Reach of Prostate Cancer

Cancer is the most deadly of modern diseases. The American Cancer Society has revealed that cancer has taken over heart disease as the number one cause of death in USA citizens under 85. In 2002, 476,009 USA citizens under 85 died from cancer compared to 450,637 from heart disease. It is estimated that 565,650 people will die in the USA from cancer in 2008. This is because deaths from heart disease are reducing at a greater rate than deaths from cancer (586,8 per 100,000 in 1050 to 211,1 in 2005 for coronary disease, while 193,9 to 183,8 for cancer).[2]

Roughly one of four people die from cancer in the USA.[7] The greatest reduction in cancer deaths in the past 10 years has been due to greatly reduced numbers of smokers, but better screening methods and newer treatments have also helped. The American Cancer Society claims that a third of all cancer deaths are due to smoking and another third is due to obesity and poor diet. The best way to avoid dying from cancer is to have a healthy diet, exercise, not smoke and have regular health checkups. Also, African-american people are in higher risk of cancer than White people, followed by Hispanics, Asians and Native-americans.

Although roughly 52% of all new estimated cases for 2008 are attributed to the male population (745,180 new cases) and 48% to the female population (692,000 new cases), lifetime probability of developing cancer between 2002-2004 is 1 in 2 for men and 1 in 3 for women.

The leading cancer type in the male population is prostate cancer. 1 of 6 men will develop prostate cancer (25% of all newly-developed cancers) during their lifetime, preceded by 1 in 3 for lung and bronchus cancer and 1 in 18 for colon and rectum. For women, leading cancer sites are breast (1 in 8, 26% of all newly-developed cancers), lung and bronchus (1 in 16) and colon and rectum (1 in 19).

Furthermore, prostate cancer is the second leading cause of death after lung and bronchus between other cancer types. 294,120 men and 271,530 women are expected to die from cancer in the USA alone during 2008. This explains from a 31% of all male cancer deaths from lung and bronchus, succeeded by 10% from prostate cancer and 8% from colon and rectum. For the female population, leading cancer death rates are from lung and bronchus (26%), breast (15%) and colon and rectum (9%).

This high death rate attributed to prostate cancer is underestimated when looking at the 5-year survival rates for prostate cancer (99% for 1996 to 2003) compared to other kinds of cancer (overall cancer: 66%, colon and rectum: 66%, lung and bronchus: 16%, pancreas: 5%). The reason for that is the slow nature of prostate cancer. In most men with prostate cancer, the disease grows very slowly. The majority of men with low-grade, early prostate cancer (which means that cancer cells have been found only in the prostate gland) live a long time after their diagnosis. The belief that people usually do not die from prostate cancer, but rather live with it until they eventually die of some other, unrelated cause, has two sides. It is true, that a small percentage of the people with prostate cancer (15-20%) will die from it if compared to other, more aggressive cancers. But still, it is the second leading cause of death among cancers.

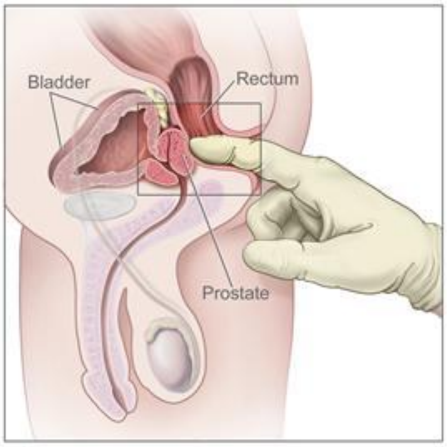
The most important risk factor for prostate cancer is age; more than 70 percent of men diagnosed with this disease are over the age of 65. Rates of prostate cancer vary widely across the world. Although the rates vary widely between countries, it is least common in South and East Asia, more common in Europe, and most common in the United States. There is some evidence that dietary factors are involved, such as vitamin E and selenium, which may have a protective effect. Genetic factors also appear to play a role, particularly for families in which the diagnosis is made in men under age 60.

About 90% of prostate cancers are diagnosed at an early stage. Nevertheless, the treatment selection is very difficult. Since prostate cancer is predominantly a disease of older men, many will die of other causes before a slowly advancing prostate cancer can spread or cause symptoms. The age and underlying health of the man, as well as the grade and stage of the disease and response of the cancer to initial treatment, are important in prognosing the outcome of the disease. The decision whether or not to treat localized prostate cancer with curative intent is a patient trade-off between the expected beneficial and harmful effects in terms of patient survival and quality of life.

I.3.b. Symptoms and Diagnosis

Neither the believed pre-cancerous condition PIN, nor most prostate cancers, cause symptoms for many years. By the time symptoms occur, the disease may have spread beyond the prostate. Even more, these symptoms are not exclusively related to prostate cancer, but to many other prostatic diseases, like BPH or prostatitis. Usual symptoms are of urinary kind, such as trouble urinating and a weak flow of urine, pain or burning during urination and needing to urinate often, especially at night. Also, difficulty having an erection, blood in the urine or semen and frequent pain in the lower back, hips, or upper thighs, are common symptoms.[8]

The absence and unspecificity of symptoms makes early screening the modality of choice. Two tests have to be performed to assure a smaller risk of not detecting prostate cancer. One is the digital rectal exam (DRE, Figure 4), in which the physician inserts a lubricated, gloved finger into the rectum to feel the size and surface of the prostate gland. Healthy prostate tissue is soft, while malignant tissue is firm, hard, and often asymmetrical or stony. The other is a blood test used to measure a protein produced by the prostate that normally is present in the blood, the prostate-specific antigen (PSA). Since the PSA level increases with age, early yearly monitoring and establishing a baseline helps in noting immediately any significant increase. Though prostate cancer may show low PSA levels, a highly elevated PSA count above 20 ng/mL is considered a strong indication of prostate cancer. But again, prostatitis and BPH may also increase PSA levels.



from http://www.med.nyu.edu/healthwise/article.html?hwid=ncicdr0000062965

Figure 4. Digital rectal examination (DRE) to feel abnormal tissues in the prostate.

Other variations of the PSA test are the PSA velocity (the rate at which serum PSA levels change over time) and the percent-free PSA or PSA II. The PSA II measures the percent of PSA that is free in the blood compared to the PSA that is bound to other proteins, so that a low percentage of free PSA (even if the total PSA is not especially high) may mean that prostate cancer is more likely. The fact that approximately 90% of all prostate cancers in the USA are currently diagnosed at an early stage relies on the widespread implementation of PSA testing.

If a man's PSA level is elevated and digital rectal exam is abnormal, the diagnosis of prostate cancer is usually confirmed by a biopsy and establishing the Gleason score. There are 2 types of biopsy procedures used to diagnose prostate cancer: the transrectal biopsy, in which tissue from the prostate is removed by inserting a thin needle through the rectum and into the prostate, and the transperineal biopsy, by inserting a thin needle through the skin between the scrotum and rectum and into the prostate. The advantage of the transrectal biopsy over the transperineal is the use of transrectal ultrasound (TRUS) to guide the needle, which is at the same time used to perform a visual examination of the gland and surrounding tissue for tumors.

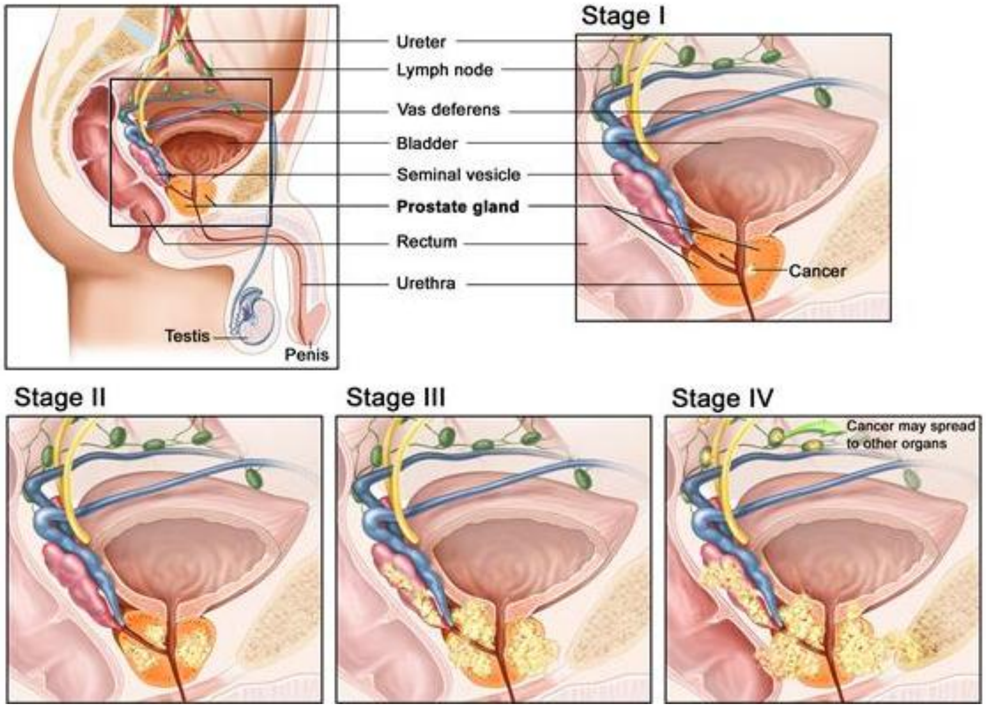
The Gleason score is a grading system used to characterize the aggressiveness of prostate cancer, which provides an estimate of the cancer's potential to grow and spread to other parts of the body. A pathologist examines two tissue samples taken from different areas of the tumor and assigns a score of 1 to 5 to each sample. The less resemblance of the cellular structure of a tumor to a normal prostatic tissue, the more likely it is to grow faster and spread outside the prostate and the higher the score. The sum of the two produces the Gleason score. Gleason scores of 2 to 4 indicate that the cells are well differentiated, whereas 8 to 10 means they are poorly differentiated.

After prostate cancer has been diagnosed, other tests are performed in order to determine the stage of the disease. These include: radionuclide bone scan, a nuclear imaging procedure that is used to detect metastasis to bones, since it is the first place where prostate cancer spreads; nuclear magnetic resonance imaging (NMRI), a computerized picture made by magnetic fields and radio waves that can show detailed, cross-sectional images of the inner body; computer-assisted or computerized axial tomography (CAT scan), an x-ray procedure that produces three-dimensional images of internal organs and glands by using contrast substances; positron emission tomography (PET), an imaging technique which uses γ-radiation and assesses particular features of the tumors biology, such as whether it is responsive to androgen suppressive agents; seminal vesicle biopsy, the removal and microscopic analysis of fluid from the seminal vesicles; and pelvic lymphadenectomy, a surgical procedure to remove the lymph nodes in the pelvis, after which the tissue is analyzed under the microscope.

The stage of the cancer is determined based on these results and on the Gleason score. The traditional 0-IV stages classification (Figure 4) has been recently replaced by a newer A-D convention:

- Stage 0: (TX, T0) no cancer is detected.
- Stage I (also called A1, T1): cancer is found in the prostate only. It cannot be felt during a DRE and is not visible by imaging. The Gleason score is low. It is usually found accidentally during surgery for other reasons, such as benign prostatic hyperplasia.
- Stage II (A2, stage B1/B2, T2): cancer is more advanced than in stage I, but has not spread outside the prostate. The Gleason score can range from 2-10.
- Stage III (stage C1/C2, T3/T4): cancer has spread beyond the outer layer of the prostate to nearby tissues and may be found in the seminal vesicles. The Gleason score can range from 2-10.
- Stage IV (D1/D2/D3, T4): cancer has metastasized to lymph nodes near or far from the prostate or to other parts of the body, such as the bladder, rectum, bones, liver, or lungs. Metastatic prostate cancer often spreads to the bones. The Gleason score can range from 2-10.

An important factor for prognosis besides the stage is, if whether the cancer has recurred or not. Recurrent prostate cancer is cancer that has come back after it has been treated. The cancer may come back in the prostate or in other parts of the body.



from http://www.med.nyu.edu/healthwise/article.html?hwid=ncicdr0000062965

Figure 5. As prostate cancer progresses from Stage I to Stage IV, the cancer cells grow within the prostate, through the outer layer of the prostate into nearby tissue, and then to lymph nodes or other parts of the body.

I.3.c. Classic and Alternative Therapies

After establishing the grade and stage of the disease, an appropriate treatment can be chosen. Due to the advanced age of over 70% of prostate cancer patients, and since 90% of prostate cancers are discovered at an early stage, the treatment of choice is watchful waiting, also called active surveillance or expectant therapy. A medical or an interventional treatment is usually recommended for high-grade cancers, while observation may be recommended for low-grade cancers, especially when the patient is elderly or has other medical conditions.[9]

Watchful waiting is an option recommended for patients with early-stage prostate cancer, particularly those who have low-grade tumors (low Gleason score, low PSA levels) with only a small amount of cancer seen in the biopsy specimen and nonpalpable tumors. Older patients and those with serious medical problems are also good candidates for active surveillance. These patients have regular examinations (DRE), PSA testing, and sometimes scheduled biopsies. If there is evidence of cancer growth, active treatment may be recommended. The risk of this treatment is that the tumor may grow between monitoring visits and spread to other parts of the body.

There are two kinds of radiation therapy: internal and external radiation therapy. They both involve the delivery of radiation energy to the prostate in order to slowly disintegrate the tumor. Brachytherapy is a minimally invasive internal radiation therapy, which involves implanting low or high dose radiation (LDR or HDR) seeds in the prostate using a needle. This therapy is usually more effective for younger patients in good health with localized prostate cancer. It has recurrence-free survival rates of 77-93%. Possible side effects are scrotal burning, incontinence and impotence. Electron beam radiation therapy (EBRT) is the most common external radiation therapy. Intensity modulated (IMRT) and 3D-conformal RT (3D-CRT) are newer noninvasive variations of EBRT. The energy is usually delivered in an outpatient setting using an external beam of radiation 5 times a week over 6-8 weeks. Success rates lie over 85% especially when used in combination with other therapies like a hormonal therapy. Side effects may include tiredness, diarrhea, frequent and burning urination and proctitis (inflammation of the anus and the lining of the rectum).

Radical prostatectomy is a surgical procedure to remove the entire prostate gland and nearby tissues, sometimes even lymph nodes in the pelvic area. It is an aggressive, major intervention (even though sometimes a milder, laparoscopic surgery may be possible), reason why it cannot be performed in elder patients. The major side effect of this operation is that not always a nerve-sparing surgery, for the nerves needed for an erection, is possible.

Since the rate of cell division in prostatic adenocarcinomas is comparable to that of healthy cells, chemotherapy is not a suitable therapy. Nevertheless, it is used as a salvage (last resort) therapy for metastatic prostate cancer to slow disease progression and postpone symptoms. The most commonly used chemotherapeutics for prostate cancer are: mitoxantrone, doxorubicin, vinblastine, paclitaxel, docetaxel, etoposide and estramustine (prostate cancer specific alkylating agent).

Cryosurgery is the use of needles to apply freezing gases in this case to kill cancerous cells in localized cancers. It is an alternative for external radiation resistant cancers and an effective primary treatment for early stage prostate cancer. The freezing and thawing processes destroy cells through dehydration, drastic changes in the pH levels, or prevention of the flow of red blood cells. Subjecting the prostate gland to freezing temperatures, specifically negative 40 degrees Celsius, also activates an anti-tumor response in the body. The procedure lasts no longer than two hours, with a possible overnight admission. Data for survival rates are not yet established, but very promising. Side effects may include pelvic pain, scrotal swelling and impotence.

Hormonal therapies aim at reducing androgen levels, hence the name androgen suppression (AS) or androgen deprivation therapies. Proof of principle was achieved already in the 1940s by C. B. Huggins, Nobel Prize for Physiology or Medicine (1966) holder, and C. V. Hodges,[10] for proving androgens as an important growth factor for prostate cancer. He did so by demonstrating in castrated and testosterone suppressed rats (treated with estrogens) the regression of an implanted prostate cancer tumor. Nowadays there are several classic and new, experimental AS approaches. The classical procedure by excellence is the surgical removal of the testes (orchiectomy). Through this invasive therapy, the production of about 90% of all androgens may be stopped. The remaining androgen production is mainly carried out in the adrenals, which is enough to keep the tumor on growing, reason why orchiectomy is usually accompanied by medical castration.

The medical castration called combined or multiple androgen blockade (CAB), also called combined or total hormone blockade, is another classic AS therapy. Commonly, CAB treatment starts with an antiandrogen (flutamide, bicalutamid: androgen receptor antagonists; finasteride, dutasteride: 5α-reductase inhibitors), a few weeks after which a gonadotropin-releasing hormone (GnRH) agonist (buserelin, goserelin) is administered. GnRH agonists themselves lead to a hormone flare which is a reaction that causes the body to flood with testosterone for 7 to 10 days. This can be painful, deadly, or both for patients with advanced prostate cancer with distant bone metastasis. Antiandrogens block the body's ability to use testosterone, so the flood of testosterone never reaches the prostate gland. This form of combined androgen blockade is common for patients in later stages of prostate cancer.

Very often after a medical or surgical castration, prostate cancer cells become resistant to an AS therapy and become so called androgen or castration-resistant, or hormone-refractory or castration refractory prostate cancer (HRPC). Antiandrogens then stimulate cancer growth, while its removal initially stops cancer from growing. This is one of the main reasons why AS therapy, being also a mild one, is usually saved as a last resort for after the patient has developed metastatic prostate cancer and become also considerably older.

A hormonal therapy, suitable for early and advanced (metastatic), as well as for HRPC, is the use of CYP17 inhibitors. By blocking the biological pathway of androgen biosynthesis at an early stage, production of testosterone and all other androgens may be stopped in the testes, adrenals and in the castration-resistant prostatic tumor, thus preventing the tumor from growing further and, according to recent studies, even delaying the development of bone metastasis for a period of up to eight years.[11]

I.4. CYP17

I.4.a. CYP Superfamily

CYP is the abbreviation for cytochrome P450. Cyto is derived from the Greek κίτος (kytos) which means a hollow, cell or container, and chrome from the Greek χρώμα (chroma), which means color. 'P450' refers to a feature of this pigment (hence 'P') in the carbon monoxide absorption difference spectrum at 450 nM caused by the presence of a thiolate in the axial position of the heme (a porphyrin complexing an iron(III) cation) in the active site, opposite to the carbon monoxide ligand. Thus, cytochrome P450 makes allusion to the 450 nM color absorbing enzyme in cells. Cytochrome P450 is a very large and diverse superfamily of hemoproteins (heme containing), member of an even wider superfamily of heme-containing monooxygenases, besides secondary amino monooxygenase – SAMO, and heme chloroperoxidase – CPO. CYPs are involved in xenobiotic (foreign bodies, drugs) metabolism (70-80% of human xenobiotic metabolism), cholesterol biosynthesis, and steroidogenesis (synthesis of steroids) in eukaryotic organisms (thus in humans) and are found mainly in the endoplasmic reticulum and inner mitochondrial membrane of cells. CYP enzymes usually contain about 500 amino acids, and more than 7700 distinct CYP sequences (thus 7700 different enzymes) are known. They have been identified from all lineages of life, including mammals, birds, fish, insects, worms, sea squirts, sea urchins, plants, fungi, slime molds, bacteria and archaea.

In the human species, 18 CYP families divided in 43 subfamilies are present, containing altogether 57 genes and 59 pseudogenes. Each gene is responsible for coding one protein, resulting thus in 57 human CYP enzymes. Families may be divided according to their biological function in: drug and steroid metabolism (CYP1, CYP2, CYP3) through redox metabolism (mainly for the excretion of xenobiotics) or anabolism (biosynthesis), and steroid anabolism (biosynthesis; CYP11, CYP17, CYP19, CYP21), while the other enzymes are involved in the metabolism of vitamins (CYP24, CYP26), cholesterol (CYP39, CYP46, CYP51), fatty acids (CYP4) or varied functions (CYP5, CYP7, CYP8, CYP27), or have even yet unknown function (CYP20).

The monooxygenase reaction, also called oxyreductase, is the insertion of one atom of oxygen into an organic substrate (RH) while the other oxygen atom is reduced to water:

$$RH + O_2 + 2H^+ + 2e^- \rightarrow ROH + H_2O$$

This sum reaction is actually a part of a catalytic cycle (Figure 6). In the normal state, the iron(III) is coordinated by the porphyrin, the thiolate of the cysteinate, which holds the heme, and a water molecule. The cycle starts with the displacement of the water molecule by the substrate (1), leading to the donation of an electron by the cofactor NAD(P)H (2), thus changing the oxidation of the ferrous iron(III) to ferric iron(II). Through addition of an oxygen molecule (3), the oxidation step changes back to (III) and a second electron coming usually from cytochrome b5 (cyb5) negatively charges the oxygen (4). A water molecule results from the successive addition of two protons, proceeding from surrounding water or amino acid residues, and resulting in a highly reactive iron(V)-oxo system (5). The oxygen then inserts between the R-H bond of the substrate (6), thus returning the electronic configuration of the iron back to (III) which is stabilized again by a water molecule.

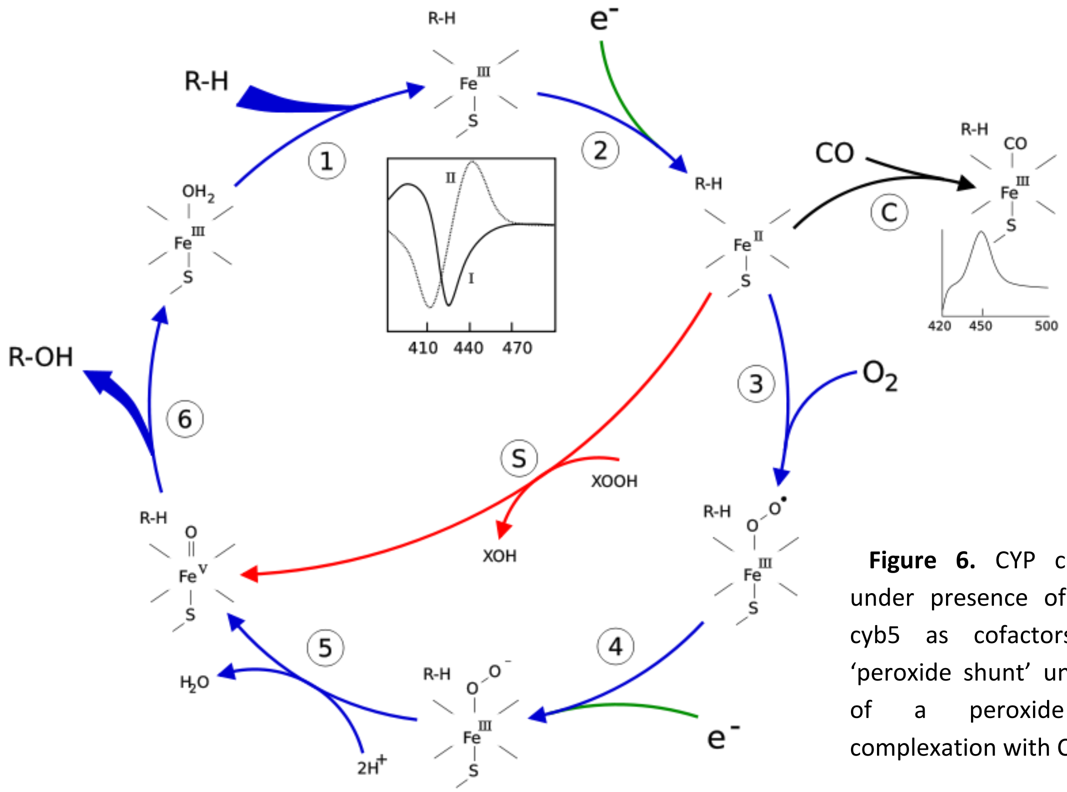


Figure 6. CYP catalytic cycle under presence of NADPH and cyb5 as cofactors (1-6), the 'peroxide shunt' under presence of a peroxide (S) and complexation with CO_2 (C).

from http://commons.wikimedia.org/wiki/Image:P450cycle.svg

I.4.b. Steroidogenesis and Physiological Role

While drug metabolism is mainly regulated by CYP enzymes, mineralo- and glucocorticoids, and sex steroids are also mainly synthesized by CYP enzymes. Cytochrome P450 17α-hydroxylase/17, 20-lyase (17α-hydroxylase and 17, 20-lyase refer to the catalytic properties of the enzyme to hydroxylate the carbon 17 in α position and to cleave the bond between carbon 17 and 20), or CYP17 for short, is the key enzyme in the biosynthesis of sex-steroids (Figure 7). It is a microsomal P450 system anchored to the membrane of the endoplasmatic reticulum and consists of 467 amino acids. CYP17 is located in membranes of the adrenal cortex, testis and ovary. Bound to the membrane next to it are the electron-transferring cofactor cyb5 and the P450 reductase, which on its part transfers an electron from the cofactor NADPH, as explained in the common mechanism for the monooxigenase or hydroxylase activity of CYP enzymes (Figure 6). Although the exact mechanism for the lyase step is yet unknown, it is believed, that it follows the tetrahedric nucleophillic addition (S_{Nt}) of the iron(III)-bound oxygen radical to the carbon 20, resulting in a tetrahedric intermediate, for the steroid to leave it then through the cleavage of the 17-20 bond.

Making use of its hydroxylating properties, CYP17 catalyzes the conversion of progesterone and pregnenolone into the corresponding 17α-hydroxy intermediates, as well as corticosterone into cortisol and corticosterone into cortisol (Figure 7). Through its lyase abilities, it yields the C19 androgens (they have only 19 carbons) androstenedione and dehydroepiandrosterone (DHEA) from their respective hydroxylsubstrates. Androstenedione and DHEA are transformed into each other through the catalytic properties of 3beta-HSD. From there on, estrone and testosterone are synthesized from androstenedione, while the most potent estrogen and androgen, estradiol and dihydrotestosterone, both derive from testosterone. By inhibiting CYP17, the production of all androgens (male hormones) and estrogens (female hormones) is stopped. In the adult or senior man, this blockade has minor side effects regarding the suppression of estrogens as well as androgens. The apparent obstruction of cortisol production by CYP17 inhibition can be overpassed through alternate pathways (Figure 7), while CYP17 inhibitors have demonstrated not to lower cortisol plasma concentration in clinical trials, but to only bring it back to normal values in Synacthen (induces cortisol production) pre-treated patients. Besides, the 50-100-fold elevations of corticosterone have enough weak glucocorticoid activity to prevent glucocorticoid deficiency and adrenal crisis. Furthermore, the cortisone-cortisol equilibrium also prevents the absence of cortisol.

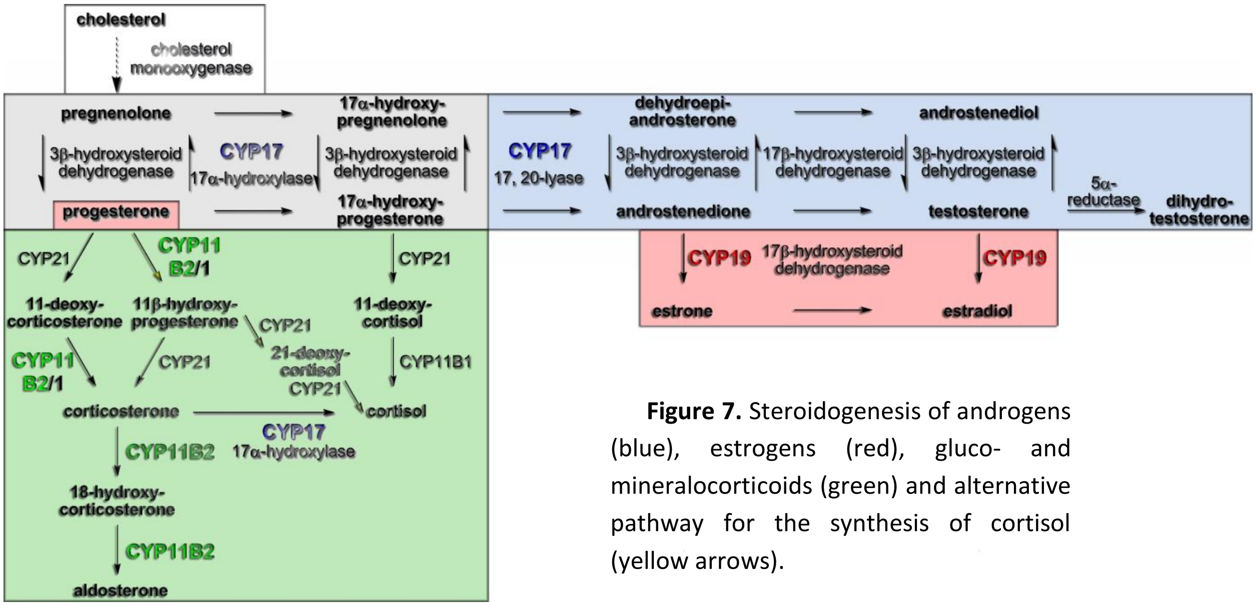


Figure 7. Steroidogenesis of androgens (blue), estrogens (red), gluco- and mineralocorticoids (green) and alternative pathway for the synthesis of cortisol (yellow arrows).

C. B. Huggins and C. V. Hodges showed in the 1940s that surgical castration leads to the inhibition of tumor growth in rats, which was attributed to the lower testosterone levels, induced years later in another test through the application of estrogens.[10] F. Labrie, the founder of CAB, hypothesized in the 1980s that additionally counteracting adrenal androgens by application of antiandrogens would further inhibit tumor growth.[12] This approach has been widely used in the past and has shown partially positive results, especially in patients with minimal disease and good performance status.[13] Nevertheless, notable side effects are observed, such as heart disease and development of androgen-resistant metastases for which this treatment is not suitable anymore.[14]

Testicular, adrenal and tumor *in situ* androgen level suppression may be achieved by other approaches, such as targeting CYP17.

I.4.c. Novel Target for Prostate Cancer: State of the Art

Many are the facts which account for the advantage of a CYP17 inhibitory therapy. As already mentioned, this mild therapy is best suited for patients of advanced age, as is the case of most prostate cancer patients. Since all prostate cancers are androgen-dependant at the beginning, and most of them are diagnosed at an early stage, this could be the best option as initial treatment for preventing their growth and spreading, thus shrinking the tumor. Because of the gentle nature of the AS therapy and the good results in controlling advanced cancers, and also because of the slow cell-division rate of prostate cancer, the treatment of choice is watchful waiting to save the AS therapy for when the cancer has advanced (and the patient is much older, thus more difficult to be treated). Sometimes during the normal development of the disease, as well as after most AS therapies, prostate cancers metastasize and become resistant to current AS therapies. This is believed to be due to the storage of androgens in the tumor and to the natural mutation of the tumor cells to produce androgens themselves. Since CYP17s expression is up-regulated in such castration-resistant metastases, its inhibition should be the best method to further lower androgen levels *in situ* in such cells.[15] Summarizing, CYP17 inhibitory therapy is not only suited but also apparently the best option for treating prostatic adenocarcinomas of any grade and stage.

Proof of principle was achieved by ketoconazole (Figure 8), the first CYP17 inhibitor to be used off-label for the treatment of prostate cancer. Because of the external mode of use of this antimycoticum, selectivity issues for an internal use were not considered. Due to its unselective inhibition of many different CYP enzymes, this drug was considered to be too toxic for systematic use and its usage discontinued.[16]

Despite of the market's low rentability for the development of new prostate cancer drugs, due to the advanced age of the patients, the slow nature of the disease, and the excuse of the high 5-year survival rates (it is the second leading cause of death among cancers due to the high occurrence and recurrence of prostate cancer), some industrial companies (Cougar Biotechnology, Takeda and others) and academic research groups (Hartmann, Brodie, Miller and others) investigate in the CYP17 inhibitory field. In this way, abiraterone (Figure 8) was developed by Cougar Biotechnology, and is recently undergoing phase III clinical trials.[17] It shows high activity in post-docetaxel HRPC patients, produces no alteration of cortisol levels and, in contrast to ketoconazole, it seems to have no dose-limiting toxicity. The main drawback of this drug is its steroidal scaffold, as steroidal compounds often show side effects due to interactions with steroid receptors.

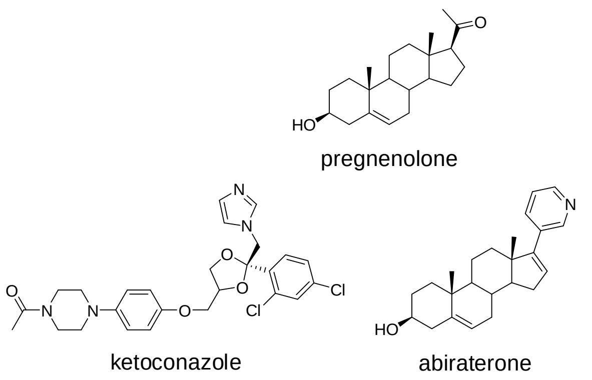


Figure 8. Pregnenolone as CYP17 substrate for androgen biosynthesis, ketoconazole as unselective non-steroidal CYP17 inhibitor, abiraterone as substrate-derived CYP17 inhibitor.

II. Aims and Strategy of this Work

II.1. Problematic and Aims

Prostate cancer is one of the major causes of death in the male population. Relying on its mildness, effectiveness and clinical experience, the most promising prostate cancer therapy is androgen suppression (AS). One drawback of surgical and medical AS treatment is their limited and insufficient inhibition. Besides, another main drawback of medical AS is that current antiandrogens stimulate the development of hormone-refractory prostate cancer (HRPC). Those are the reasons why AS is not made use of for early cancers, even if it has been shown to relay bone metastasis for eight years in early cancers. This drawback should be overcome by CYP17 inhibitors, which should be equally and even more effective in HRPC. Since CYP enzymes are the main responsibles in xenobiotic metabolism, hepatotoxicity is a main issue in the development of such inhibitors. One steroidal CYP17 inhibitor is currently in clinical trials, while others are being developed. Nevertheless, steroidal drugs usually account for several side effects.

The sequence identity and sequence homology between CYP enzymes is usually very high. Furthermore, they have a very similar secondary protein folding, thus very similar binding sites. In such manner, the selectivity issue becomes even much more critical while trying to develop CYP17 inhibitors, which should inhibit the enzyme of transforming the substrate by blocking its binding site. Furthermore, the substrates for the sex and mineralo- and gucocorticoid forming enzymes bear all a steroidal scaffold and are respectively very similar. All these similarities increase furthermore the relevance of developing nonsteroidal inhibitors in order to achieve selectivity towards CYP enzymes.

These selectivity problems and the fact, that CYP17 is a vital enzyme, makes the use of suicide inhibitors, that means inhibitors which bind covalently, thus irreversibly, to the enzyme, unacceptable.

In such manner, our aims are the design, synthesis and evaluation of potent and selective nonsteroidal competitive CYP17 inhibitors, which should complex but not covalently bind to the substrate’s binding site.

II.2. Working Strategy

II.2.a. Molecular design

In this work, a classical medicinal chemistry approach was used. Starting from compounds with known CYP17 activity, other core structures were designed and synthesized, and evaluated step by step regarding their potency and selectivity (‘screening’), in order to repeteadly optimize them, through small structural variations, in terms of potency and selectivity toward other therapeutical targets.

In such manner, starting from biphenylic compounds (Figure 9), aromatic and heteroarylic annulations to both benzene rings were made, leading to two major compounds classes: ABD- and ACD-ring substrate mimetic naphthalenes as CYP17 inhibitors. From there on, through replacement of the D-ring with a more similar to the substrate 5-membered aryl, and making use of the knowledge gained about functional groups interacting with our enzyme which was reaffirmed by modeling studies, abiraterone analogues were achieved as highly potent and selective CYP17 inhibitors.

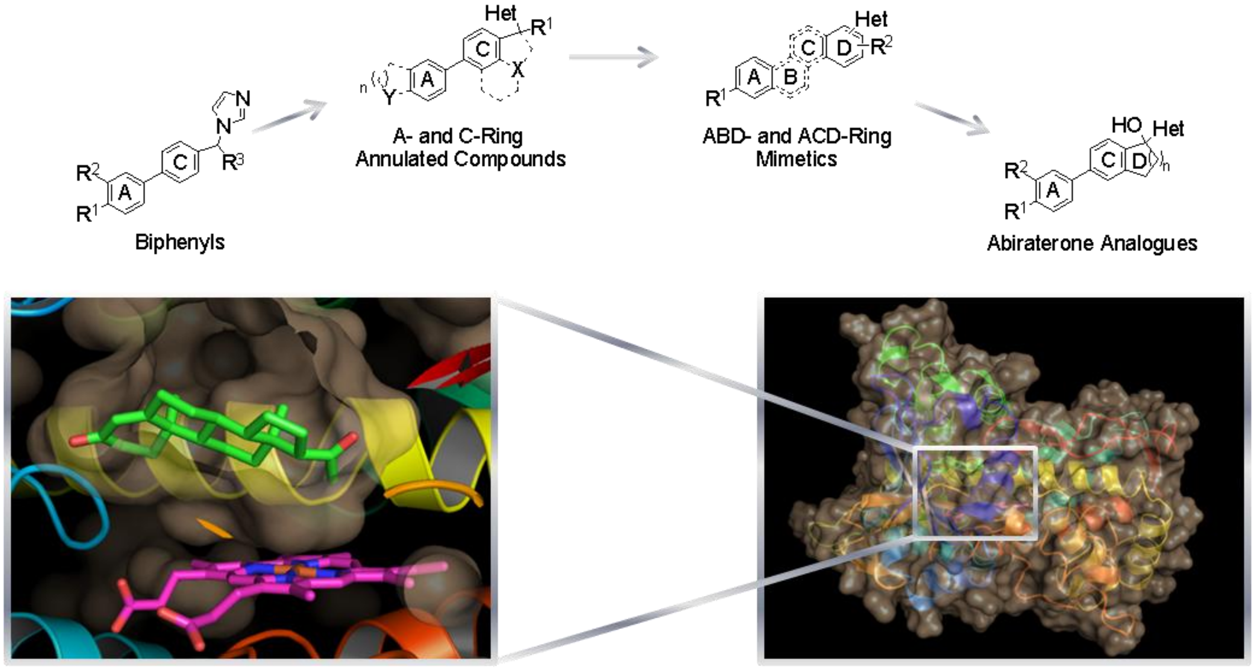


Figure 9. Designed, synthesized and evaluated classes, developed through the medicinal chemistry approach used, and CYP17’s binding site from our enzyme model. In light green, pregnenolone as substrate; in magenta, the enzyme’s heme; and in red, dark green, blue, yellow, orange and cyan, the enzyme’s tertiary structure.

Subject of this work are the mentioned classes of the annulated biphenyls, ABD- and ACD-ring mimetics and abiraterone analogues as CYP17 inhibitors and pyridyl-substituted naphthalenes as dual inhibitors of CYP17 and CYP11B2 published in three major scientific journals with the primary author listed as first author and some other also highly potent compounds belonging to similar classes, synthesized by the same person, and mentioned before the introduction of this work.

Very important for inhibiting a binding site is to have the certainty of how a compound will lie in the pocket, and in which direction it will orient itself. Thus, the key consideration for inhibiting a CYP enzyme's binding site is in relation to their common feature: the heme. Substrates will orient towards the heme, since the catalytic reactions are performed in that region. Because of that, the heme iron is a good anchor point for blocking the binding site, fixing the inhibitor in the catalytic region of the enzyme. sp^2 hybridized nitrogen atoms are well known to be very good at complexing iron(III) ions, and many N-bearing aromatic heterocycles are described, pyridine undergoing one of the best coordinations.

Other important characteristics of the substrates to be considered, in order to maximally increase the chance of our compounds to lie in the same manner as them, are the lipophillity, and planarity and rigidity of the steroidal backbone. This is successfully maintained in the developed aromatic systems, leading to good inhibitory results.

A third characteristic germane to the substrates is the oxygen functionality at the carbon 3 position. This was also to be carried over to our systems through the introduction of different electron donor and acceptor groups.

II.2.b. Biological Evaluation

In order to evaluate and successfully identify potential therapeutic drugs, our well-established screening system (Figure 10) was used. After the design and synthesis of prospective candidates, they were initially tested for their CYP17 inhibitory potency using our *in vitro* human CYP17 assay, by incubating the microsomal fraction of our recombinant *E. coli* cells stably expressing human CYP17. The products were detected and quantified by means of UV-spectroscopy.

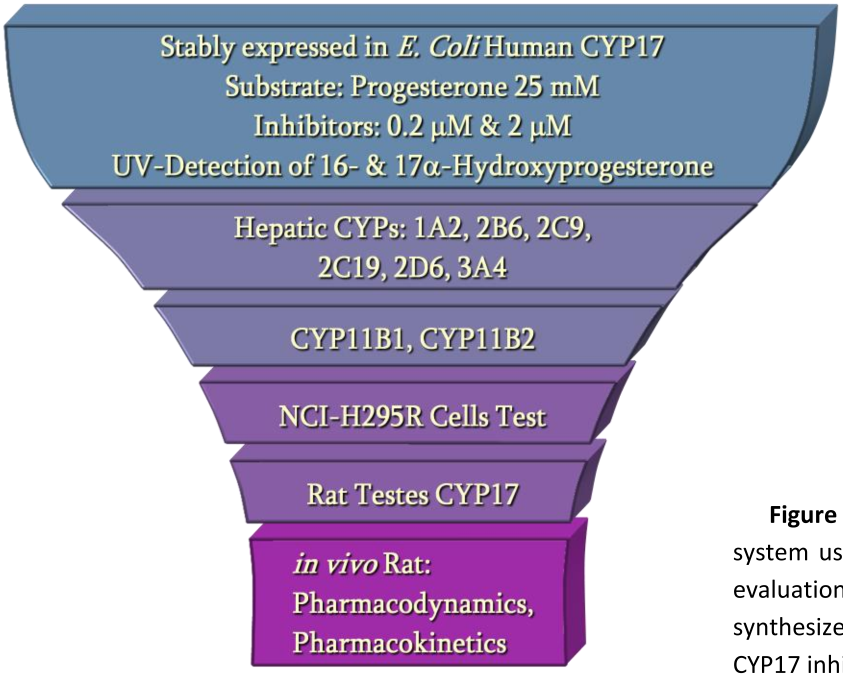


Figure 10. Screening system used by us for the evaluation of the synthesized compounds as CYP17 inhibitors

Highly active and promising compounds were subsequently tested for hepatotoxicity on different naked hepatic enzymes (BD Biotechnologies, Supersomes™), such as CYP3A4, responsible for over 50% of xenobiotic metabolism, CYP2D6 and CYP1A2, which show pronounced genetic polymorphism and other enzymes, CYP2B6, CYP2C9 and CYP2C19, all relevant to xenobiotic metabolism, and which constitute altogether more than 90% of drug metabolism.

Later on, highly active, non-toxic compounds were evaluated for inhibitory activity on human CYP11B2 and human CYP11B1, using respective recombinant V79 cells for each enzymatic assay. The assays were performed analogously, as previously described by us. The importance of testing our compounds for selectivity in both enzymes relies on the facts described above: similarity between CYP17, CYP11B2 and CYP11B1 (chapters I.4 and I.5).

Selected, highly promising compounds are to be tested later on in an NCI-H295R (a human adrenocarcinoma cell line) cells test, expressing CYP17, CYPscc, CYP11B2, CYP11B1, CYP21, and many other relevant enzymes, and thus resembling a self-consistent *a priori* steroidogenetical model suitable for predicting the overall influence of the test compounds in a more complex environment similar to a cancerous tumor. Following that test, compounds are to be evaluated in a rat testes test, allowing to observe, between other important factors, if the designated compounds show similar activity on the rat enzyme, before testing them in the living animal. This has to be checked before, since there are differences between the enzymes of both species with underlying 68% sequence identity and 82% homology. The final pre-clinical step performed by us is the *in vivo* evaluation in the rat and eventually in other animals, for the determination of pharmakokinetics and -dynamics.

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

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Arch. Pharm. Chem. Life Sci. **2008**, *341*, 597–609

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.

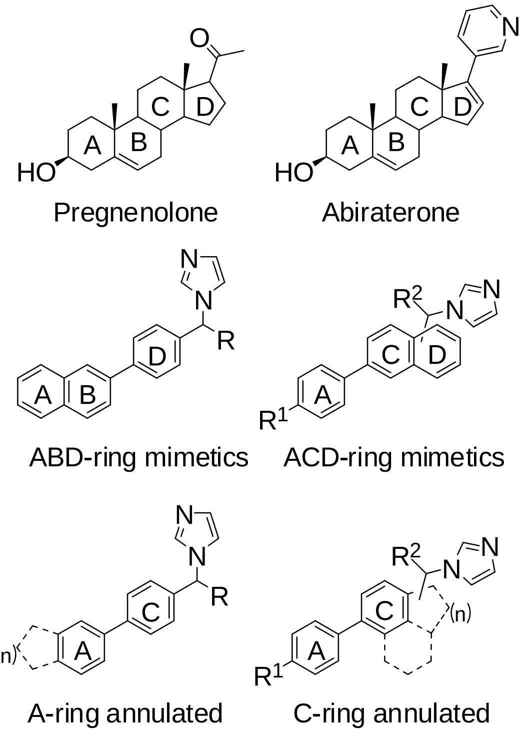
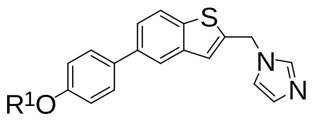


Chart 1. Substrate, abiraterone, described ABD- and ACD-ring mimetic CYP17 inhibitors and A- and C-ring annulated compounds of the present study.

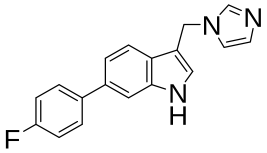
Very recently,[12] we found new highly potent and selective compounds, which showed better pharmacokinetic and pharmacodynamic profiles than abiraterone, a CYP17 inhibitor currently undergoing clinical phase II,[13] by replacing the A-ring-mimicking benzene nucleus with different heterocycles.

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**). 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.



1, 2



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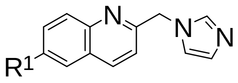
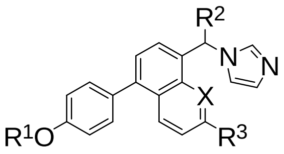
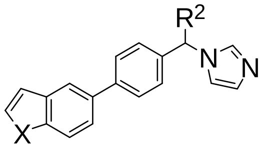


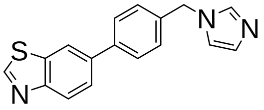
4, 5



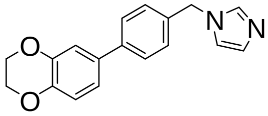
6-11



12-16



17



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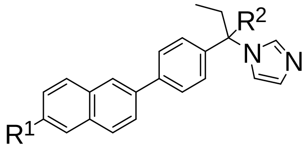


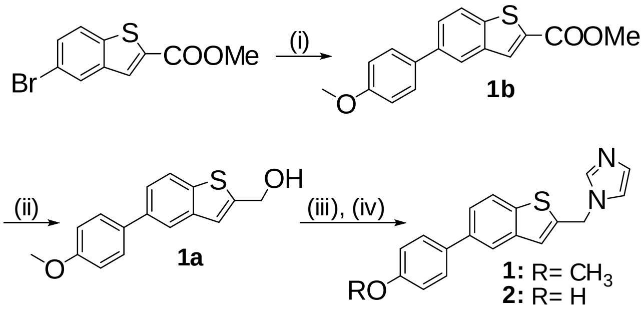
19-21

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 synthesised compounds **1-21**.

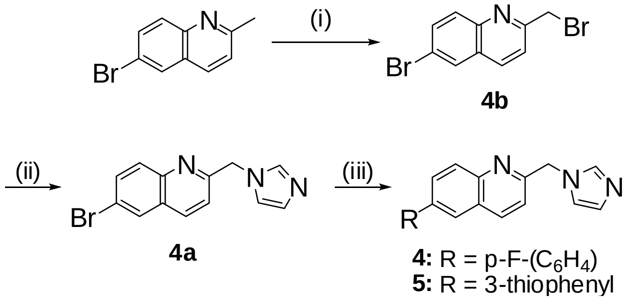
Chemistry

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]



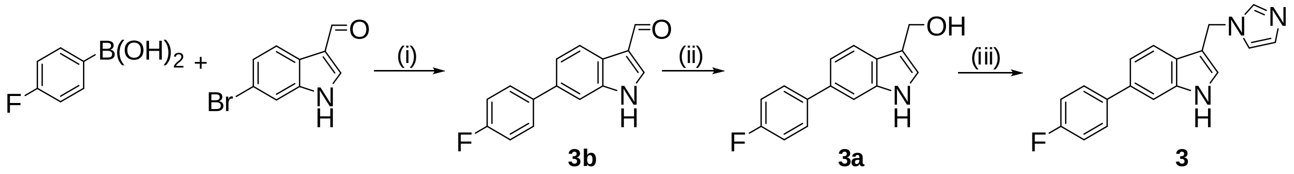
Scheme 1. Synthesis route of compounds 1 and 2

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.



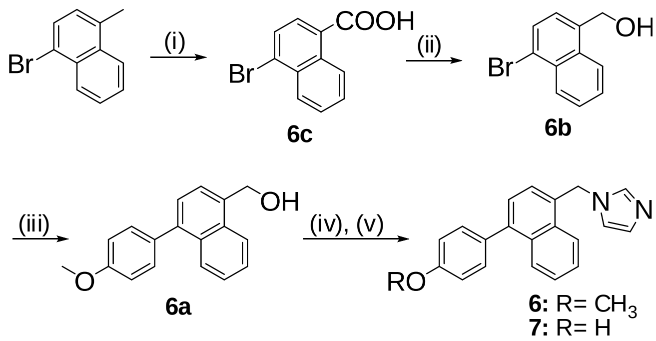
Scheme 3. Synthesis route of compounds 4 and 5

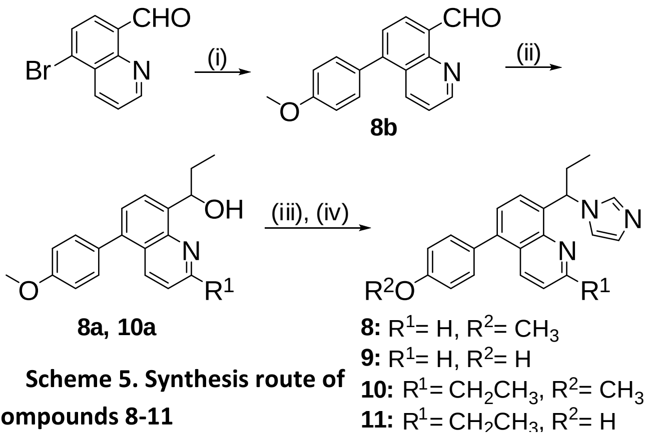
Reagents and conditions: **(i)** NBS, CCl_4, 75°C, 16 h; **(ii)** Imidazole, K_2CO_3, 18-crown-6, acetonitrile, 100°C, 16 h; **(iii) Method C1:** 4-fluorophenylboronic acid (**5:** 3-thiophenylboronic acid), Na_2CO_3, $Pd(PPh_3)_4$, toluene/MeOH/H_2O, 70°C, 5 h.



Scheme 2. Synthesis route of compound 3

Reagent 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.





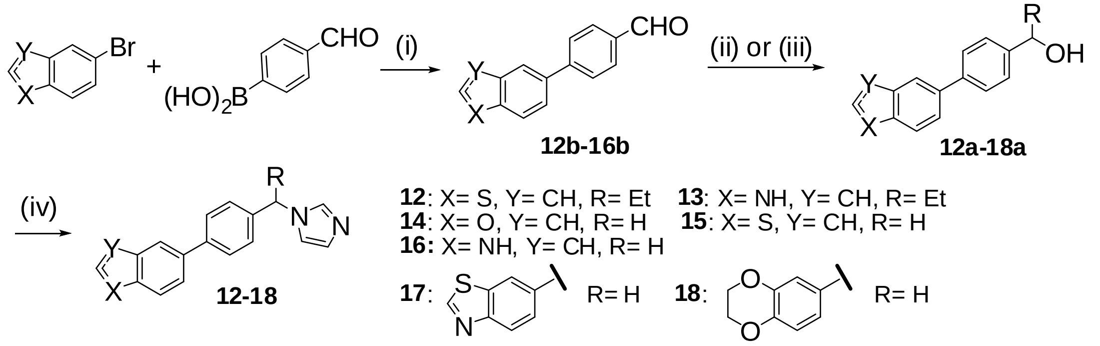
Scheme 4. Synthesis route of compounds 6 and 7

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

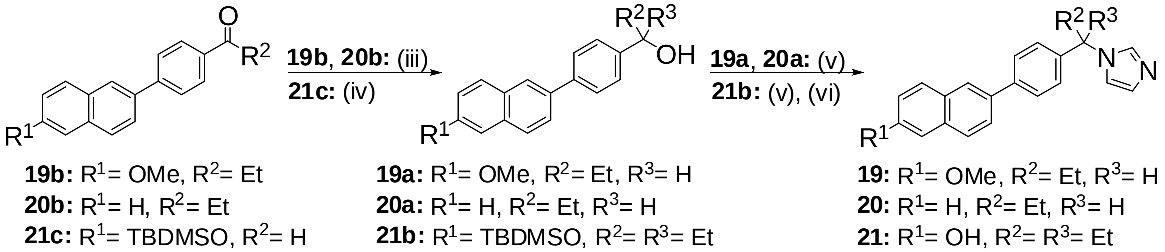
Reagents and conditions:

(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.



Scheme 7. Synthesis route of compounds 19-21

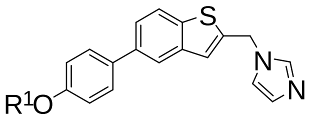
Reagents and conditions: (i) TBDMSCl (*tert*-Butylchlorodimethylsilane), CH_2Cl_2, imidazole, rt, 4 h; **(ii) Method C1:** boronic acid, Na_2CO_3, $Pd(PPh_3)_4$, toluene/MeOH/H_2O, 70°C, 5 h; **(iii) Method D: 19b**, **20b:** $NaBH_4$, MeOH, reflux, 2 h; **(iv) Method E: 21c:** EtMgBr, THF, rt, 5 h; **(v) Method A1: 19a**, **20a:** CDI, NMP, 170°C, 7 h; **(vi) 21b:** TBAF, THF, rt, 4 h.

Results

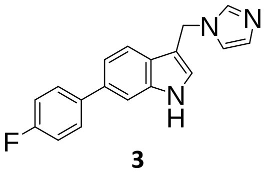
Biological Results. Inhibition of CYP17 was evaluated using human enzyme expressed in *E. coli*.[17] The percent inhibition values of the compounds were determined with the 50,000 g sediment of the *E. coli* homogenate, progesterone (25 µM) as substrate and the inhibitors at concentrations of 0.2 and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

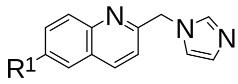
Table 1. Inhibition of CYP17 by C-ring annulated compounds **1**-**11**



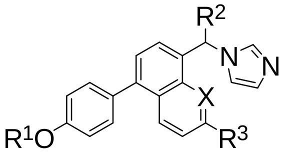
1, **2**



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.

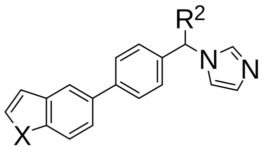
[b] **KTZ**: ketoconazole.

[c] % inhibition at 1.0 µM.

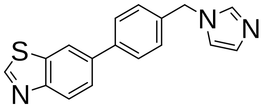
[d] IC_{50}= 817 nM

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.

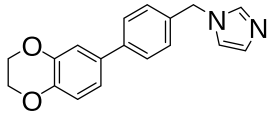
Table 2. Inhibition of CYP17 by A-ring annulated compounds **12**-**21**



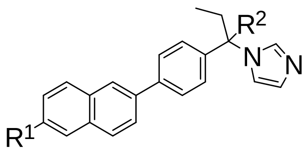
12-16



17



18



19-21

Comp.	Structures			CYP17	
				% Inhibition [a]	
	R^1	R^2	X	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.

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.

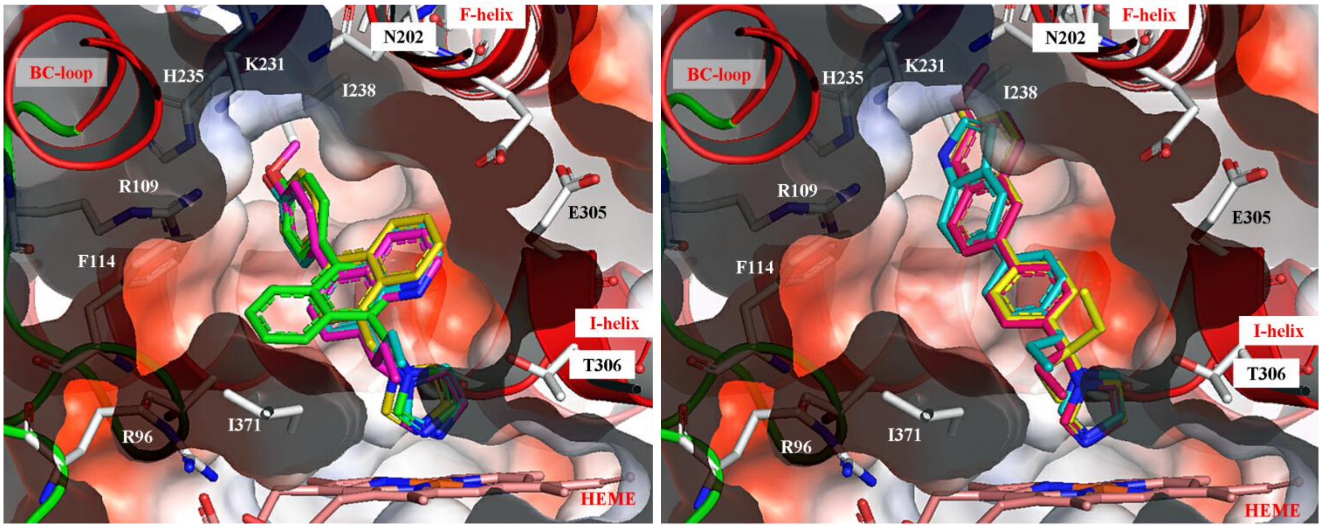
Regarding selectivity against other CYP enzymes, a broader spectrum of our compounds was tested for inhibition of the hepatic enzyme CYP3A4. This enzyme is responsible for the metabolism of lipophilic substances and, therefore, responsible for about 50% of current prescription drugs.[18] While compounds **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).

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)-1H-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,^{4} J_{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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IV. Synthesis, Biological Evaluation and Molecular Modelling Studies of Novel ACD- and ABD-Ring Steroidomimetics as Inhibitors of CYP17

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

Abstract

Two novel classes of non-steroidal substrate mimetics were synthesised and examined for their potency as inhibitors of human CYP17. Selected compounds were tested for inhibition of hepatic CYP enzymes 3A4, 1A2, 2C9 and 2C19. The most promising compound **15** showed a good inhibition of the target enzyme (31% and 66% at 0.2 and 2 µM, respectively), and little inhibition of the most important hepatic enzyme CYP3A4 (6% and 19% inhibition at 0.2 and 2 µM, respectively) and the key enzyme of glucocorticoid biosynthesis CYP11B1 (3% and 23% inhibition at 0.2 and 2 µM, respectively). Docking studies revealed that this compound does not assume the same binding mode as steroidal ligands.

Introduction

Prostate cancer is the most common disease and age-related cause of death in elder men worldwide.[1] Since it is in over 80% of the cases androgen dependent, the standard treatment is orchiectomy or its medicinal equivalent the chemical castration by gonadotropin-releasing hormone analogues, which reduce the testicular androgen production.[2] Because these treatments do not affect adrenal androgen production, they are frequently combined with androgen receptor antagonists (flutamide, cyproterone acetate) to reduce the stimulatory effects of the remaining androgens.[3] However, due to mutations in the androgen receptor, anti-androgens might be recognised as agonists,[4,5] making this so-called 'combined androgen blockade' therapy not suitable for all patients.

The antimycotic ketoconazole has proven itself clinically as a good adjuvant therapy by reducing testosterone biosynthesis through inhibition of CYP17.[6,7] Nevertheless, the toxicity drawbacks it showed have forced to suspend it from use.[8] On the other hand, the steroidal CYP17 inhibitor abiraterone (Fig. 1) passed phase II clinical trials showing high activity in post-docetaxel castration refractory PC patients and seems to have no dose-limiting toxicity.[9]

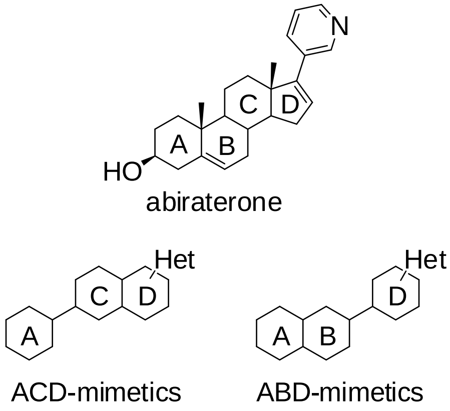


Figure 1. The steroidal CYP17 inhibitor abiraterone and ACD- and ABD-ring mimetics.

Het: N-containing heterocycle.

All this makes CYP17 an interesting target, since it catalyses both the 17a-hydroxylation of pregnenolone and progesterone and the subsequent 17,20-lyase reaction cleaving the C17-C20 bond to yield the 17-keto androgens androstendione and dehydroandrostendione, the precursors of testosterone (Fig. 2).[10] We also developed highly active steroidal inhibitors, which showed up to 3-fold higher activities against human CYP17 than abiraterone *in vitro*.[11] In order to selectively inhibit CYP17 without the potential side effects of steroidal drugs[12] non-steroidal substrate mimetics have been prepared before.[12-16] In this work, in order to find a new core structure, two different clases of substrate analogues were synthesised (Fig. 3) which mimic the A-, C- and D-rings (compounds 1-11), and the A-, B- and D-rings of the substrate with benzene nuclei (compounds 12-17). Different nitrogen bearing heterocycles were introduced at different positions, since the heme complexation by an aromatic nitrogen is an important prerequisite for a high binding affinity.[17] From previous work,[14-16] it was known that the introduction of a fluorine in the A-ring strongly contributed to a better inhibition of our target enzyme. Hydroxyl groups were introduced, too, in order to mimic the oxygen functionality of the steroidal substrates. In the following, the synthesis, biological activities and molecular modelling studies are presented. Besides the CYP17 activity, inhibition of other CYP enzymes was examined, that is, selectivity towards hepatic CYP enzymes and CYP11B1 was determined, since the latter is the key enzyme in glucocorticoid biosynthesis. The most promising structure was docked into our protein model, and the key interactions with the enzyme were elucidated.

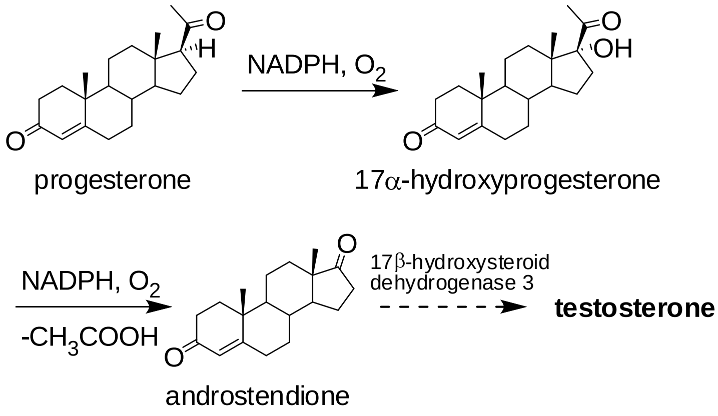
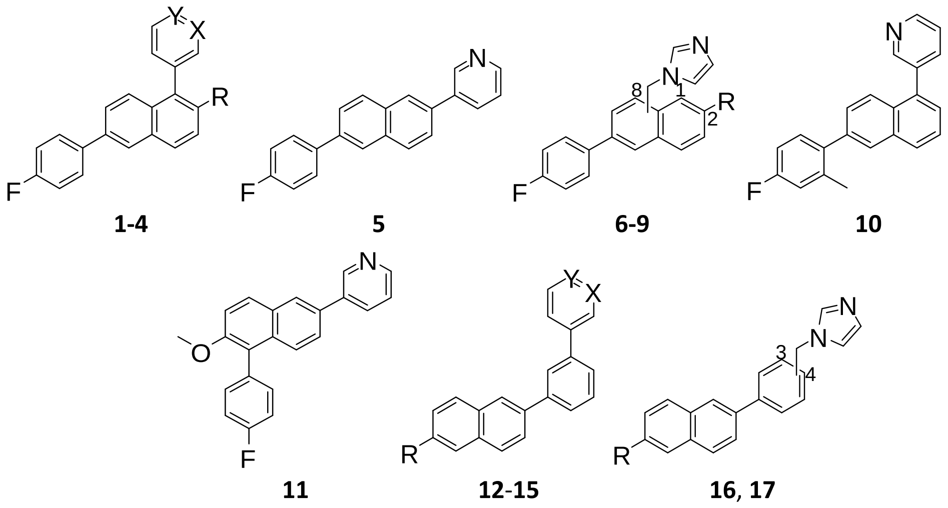


Figure 2. The role of CYP17 in androgen biosynthesis.

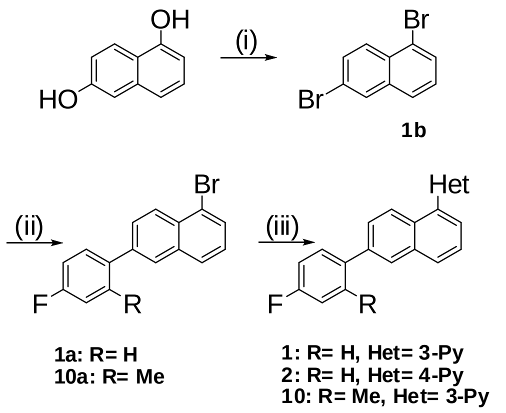


Compound	R	X	Y	subst.
1	H	N	CH	
2	H	CH	N	
3	OMe	N	CH	
4	OH	N	CH	
6	H			1
7	OMe			1
8				2
9	H			8
12	OMe	N	CH	
13	OMe	CH	N	
14	OH	N	CH	
15	OH	CH	N	
16	OMe			3
17	OH			4

Figure 3: List of synthesised compounds **1**-**17**.

Chemistry

The syntheses of compounds **1**-**17** are shown in Schemes 1-9. In our aim to find the correct pattern for progesterone mimetics, different core structure alignments were synthetised, hence the diverse synthetical pathways.



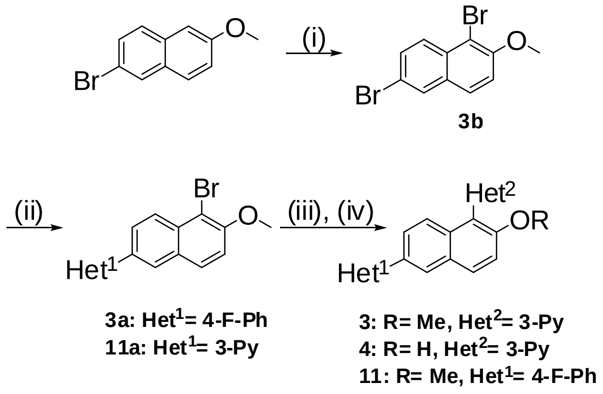
1b

1a: R= H
10a: R= Me

1: R= H, Het= 3-Py
2: R= H, Het= 4-Py
10: R= Me, Het= 3-Py

Scheme 1.

Reagents and conditions: (i) Br_2, PPh_3, acetonitrile, µw, 240°C, 10 min; **(ii) Method A: 1a:** 4-fluorophenylboronic acid (**5a:** 2-methyl-4-fluorophenylboronic acid), Na_2CO_3, $Pd(PPh_3)_4$, toluene, 110°C, 16 h; **(iii) Method A: 1, 5:** 3-pyridineboronic acid, Na_2CO_3, $Pd(PPh_3)_4$, toluene, 110°C, 16 h, **for 2:** 4-pyridineboronic acid, $NaHCO_3$, $Pd(PPh_3)_4$, DMF, H_2O, µW, 150°C, 15 min.



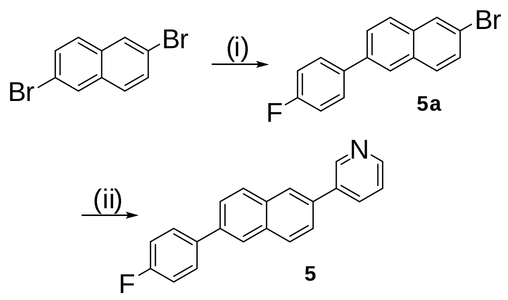
3b

3a: Het^1= 4-F-Ph
11a: Het^1= 3-Py

3: R= Me, Het^2= 3-Py
4: R= H, Het^2= 3-Py
11: R= Me, Het^1= 4-F-Ph

Scheme 2.

Reagents and conditions: (i) NBS, THF, 75°C, 2 h; **(ii) Method A: 4a:** 4-fluorophenylboronic acid (**11a:** 3-pyridineboronic acid), Na_2CO_3, $Pd(PPh_3)_4$, toluene, 110°C, 16 h; **(iii) Method A: 4:** 3-pyridineboronic acid (**11:** 4-fluorophenylboronic acid), Na_2CO_3, $Pd(PPh_3)_4$, PhMe, 110°C, 16 h; **(iv) Method B:** BBr_3, DCM, -78°C to 0°C, 16 h.



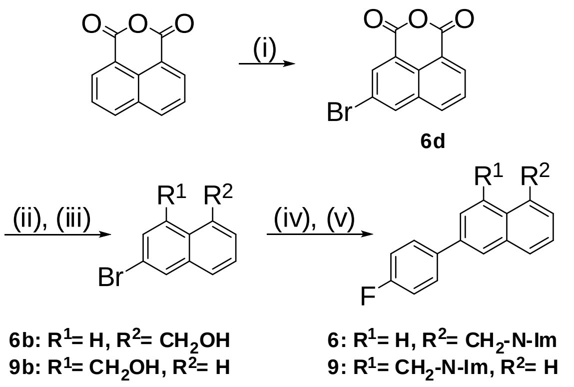
5a

5

Scheme 3.

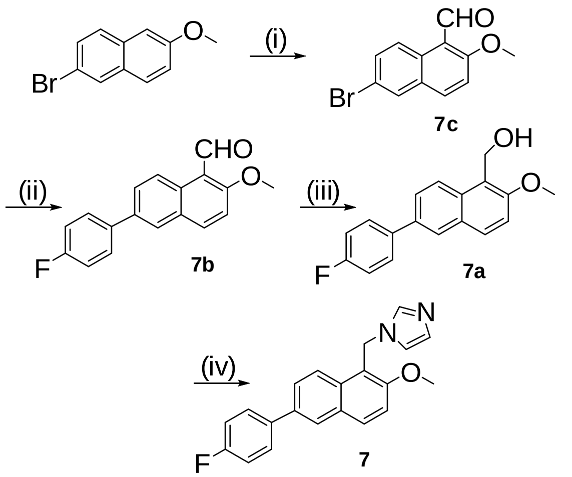
Reagents and conditions: (i) Method A: 4-fluorophenylboronic acid, Na_2CO_3, $Pd(PPh_3)_4$, toluene, 110°C, 16 h; **(ii) Method A:** 3-pyridineboronic acid, Na_2CO_3, $Pd(PPh_3)_4$, toluene, 110°C, 16 h.

The substances can be divided in pyridyl (Schemes 1, 2, 3, 7) and imidazolyl (Schemes 4, 5, 6, 8, 9) compounds. The introduction of the pyridine moiety was achieved by means of Suzuki coupling[18] (Method A), as well as the coupling of the naphthalenes and the phenyl rings. When the necessary bromides for the couplings were not commercially available, they were prepared either by bromination using NBS (Scheme 2) or with triphenylphosphine dibromide (Scheme 1). The imidazoles were introduced by performing a S_{Nt} reaction with 1,1-carbonyl diimidazole (CDI) and the corresponding alcohol[19] (Method D) in the last step. The alcohols were obtained from either the carboxylic acids (Method C) or from the aldehydes (Method E). In some cases the carbonyl group had first to be introduced (Scheme 5) or modified (Scheme 4) before reducing it to the corresponding alcohol. In some cases the methoxy-substituted compounds were submitted to an ether cleavage (Method B). For the preparation of compound **17**, the hydroxyl group on the naphthalene had to be protected before the Suzuki coupling due to otherwise very low yields.[18]



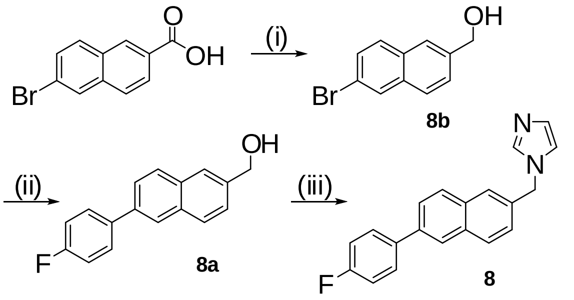
Scheme 4.

Reagents and conditions: (i) Br_2, Ag_2SO_4, H_2SO_4, 65°C, 6 h; **(ii)** NaOH, HgO, acetic acid, H_2O, 100°C, 4 d; **(iii) Method C:** $LiAlH_4$, THF, 75°C, 2 h; **(iv) Method A:** 4-fluorophenylboronic acid, Na_2CO_3, $Pd(PPh_3)_4$, toluene, 110°C, 16 h; **(v) Method D:** CDI, imidazole, NMP, 170°C, 3 h.



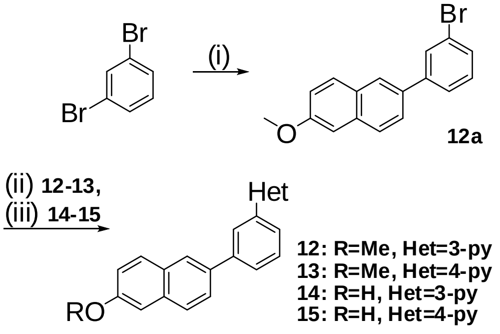
Scheme 5.

Reagents and conditions: (i) $TiCl_4$, 1,1-dichloromethylmethylether, DCM, 70°C, 2 h; **(ii) Method A:** 4-fluorophenylboronic acid, Na_2CO_3, $Pd(PPh_3)_4$, toluene, 110°C, 16 h; **(iii) Method E:** $NaBH_4$, MeOH, THF, rt, 1 h; **(iv) Method D:** CDI, MeCN, 85°C, 2 d.



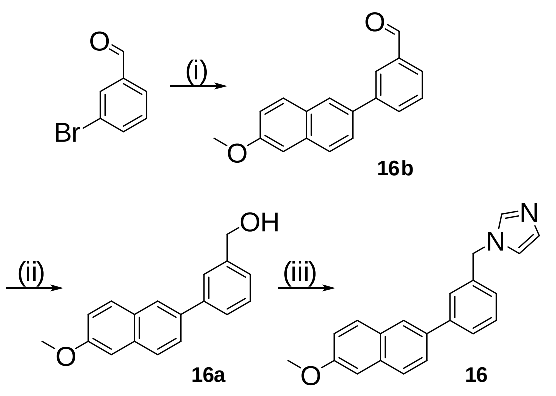
Scheme 6.

Reagents and conditions: (i) Method C: $LiAlH_4$, Et_2O, 35°C, 4 h; **(ii) Method A:** 4-fluorophenylboronic acid, Na_2CO_3, $Pd(PPh_3)_4$, toluene, 110°C, 16 h; **(iii) Method D:** CDI, imidazole, NMP, 180°C, 16 h.



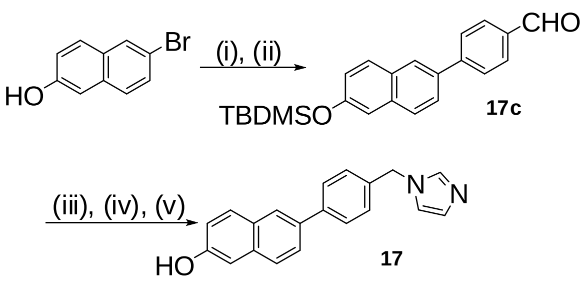
Scheme 7.

Reagents and conditions: (i) Method A: 6-methoxynaphthalen-2-ylboronic acid, Na_2CO_3, $Pd(PPh_3)_4$, toluene, 110°C, 16 h; **(ii) Method A: 12:** 3-pyridineboronic acid (**13:** 4-pyridineboronic acid), Na_2CO_3, $Pd(PPh_3)_4$, toluene, 110°C, 16 h; **(iii) Method B:** BBr_3, DCM, -78°C to 0°C, 16 h.



Scheme 8.

Reagents and conditions: (i) Method A: 6-methoxynaphthalen-2-ylboronic acid, Na_2CO_3, $Pd(PPh_3)_4$, toluene, 110°C, 16 h; **(ii) Method E:** $NaBH_4$, MeOH, THF, rt, 1 h; **(iii) Method D:** CDI, acetonitrile, 85°C, 16 h.



Scheme 9.

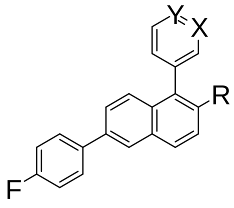
Reagents and conditions: (i) TBDMSCl, imidazole, DCM, rt, 16 h; **(ii) Method A:** Na_2CO_3, $Pd(OAc)_2$, TBAB, toluene, EtOH, H_2O, 110°C, 16 h; **(iii) Method E:** $NaBH_4$, MeOH, rt, 16 h; **(iv) Method D:** CDI, NMP, 170°C, 6 h; **(v)** TBAF, THF, rt, 16 h.

Results

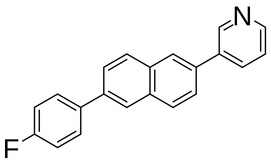
Biological Results. Inhibition of human CYP17 was determined by performing our previously described assay[20] at inhibitor concentrations of 0.2 and 2 µM. As source of human CYP17, our *Escherichia coli* system[21] (coexpressing human CYP17 and NADPH-P450 reductase) stably expressing human CYP17 was used. After homogenisation the 50,000g sediment was incubated with progesterone and NADPH as previously described.[22] Separation of the product was performed by HPLC using UV-detection.

The 3- and the 4-pyridyl-substituted ACD mimetics (**1**-**5**) showed no or little inhibition, respectively, in contrast to the reference compounds ketoconazole and abiraterone (Table 1). Amongst our methylene-imidazolyl-substituted compounds (**6**-**9**), the 8-substituted one showed the best result (**9**). It can also be observed that the introduction of a 2-methoxy group in compound **6** leads to a higher activity (**7**). Neither the introduction of a methyl substituent in the A-ring of **1** (**10**), nor the switch of the position of the A-ring from 6- to 5- (**11**) resulted in an active compound.

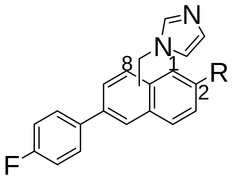
Table 1. Inhibition of CYP17 by steroidal ACD-ring mimetics (compounds **1**-**11**)



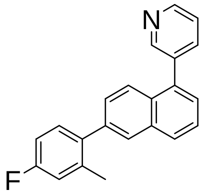
1-4



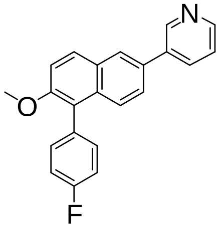
5



6-9



10



11

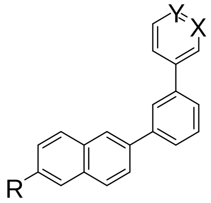
Compound	R	X	Y	subst. pos.	CYP17 % Inhibition [a,b]	
					0.2 µM	2 µM
1	H	N	CH		2	3
2	H	CH	N		1	28
3	OMe	N	CH		6	7
4	OH	N	CH		0	0
5					0	22
6	H			1	2	19
7	OMe			1	13	45
8				2	3	14
9	H			8	9	50
10					3	3
11					0	1

[a] Ketoconazole (IC_{50} = 2780 nM); abiraterone (IC_{50} = 72 nM).

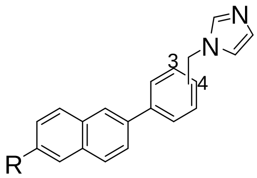
[b] Data shown are the mean of at least one independent test in duplicate. Concentration of progesterone (substrate): 25 µM. The deviations were within < ±5 %

Regarding the ABD mimetics (Table 2) it is striking that the class of the 4-pyridyl compounds (**13**, **15**) showed again activity while the 3-pyridyl compounds (**12**, **14**) were inactive. The cleavage of their methyl ether led to an enhancement of inhibition (**14**, **15**). An increase in activity for compound **13** with 23 - 66% inhibition at 2 µM (**15**) could be observed, leading to the most active compound of this study. Analogous to the work done in the ACD class, the heterocycle was replaced by a methylene-imidazole moiety, and moderate activities for compounds **16** and **17** were observed.

Table 2. Inhibition of CYP17 by steroidal ABD-ring mimetics (compounds **12**-**17**)



12-15



16, **17**

Compound	R	X	Y	subst. pos.	CYP17 % Inhibition [a,b]	
					0.2 µM	2.0 µM
12	OMe	N	CH		3	4
13	OMe	CH	N		4	23
14	OH	N	CH		0	8
15	OH	CH	N		31	66
16	OMe			3	0	28
17	OH			4	24	61

[a] Ketoconazole (IC_{50} = 2780 nM); abiraterone (IC_{50} = 72 nM).

[b] Data shown are the mean of at least one independent test in duplicate. Concentration of progesterone (substrate): 25 µM. The deviations were within < ±5 %

Since CYP3A4 is the hepatic enzyme responsible for the metabolism of lipophilic substances and therefore of about 50% of current prescription drugs,[23] a broader spectrum of our compounds were studied for their effects on this enzyme. Critical is the fact that this enzyme together with CYP2D6 and CYP1A2 shows pronounced genetic polymorphism.[24] Selected compounds (**3**, **7**, **15**, **16**) showed moderate to high activity towards the mentioned hepatic CYP enzymes (Table 3). Since compound **15** exhibited a very low activity of 19% inhibition at 10 µM towards CYP3A4[25] it was further tested on 2D6. At this enzyme, it showed inhibitions of 80% at 1 µM and 85% at 10 µM.

In further terms of selectivity, compound **15** was additionally tested on the steroidogenic CYP enzyme CYP11B1 which is involved in the glucocorticoid biosynthesis. For the assay,[26] V79MZh11B1 cells expressing human CYP11B1 were used. Compound **15** showed very low activities of 3% at 0.2 µM and 23% at 2 µM.

Table 3. Inhibition of hepatic CYP enzymes (1A2, 2C9, 2C19, 3A4) by compounds **3**, **7**, **15**, **16**

	CYP1A2		**CYP2C9**		**CYP2C19**		**CYP3A4**	
Compound	**1.0 µM**	**10.0 µM**	**1.0 µM**	**10.0 µM**	**1.0 µM**	**10.0 µM**	**1.0 µM**	**10.0 µM**
3	40	87	66	54	68	28	64	86
7	80	85	100	100	93	96	93	95
15	98	98	94	97	86	89	6	19
16	96	97	97	100	95	97	91	96
KTZ [b]	8	38	21	75	24	1	1	4
ABT [b]	36	53	17	51	3	7	7	7

[a] Data shown are the mean of three independent tests.

[b] KTZ: ketoconazole; ABT: abiraterone. The deviations were within < ±5 %

Molecular Modelling. Since there is no crystal structure of CYP17 available we built a homology model of CYP17 using the X-ray structure of human CYP2C9 (PDB code 1r9o) as template, as described before by us.[28] Docking simulations were carried out by means of the GOLD v3.0.1 software. For the docking studies, our homology model was used running Linux Suse 10.1 on Intel® P4 CPU 3.00 GHz, and the energy minimisation was also performed as previously described.[28] Compound **15** and the steroidal inhibitor abiraterone were docked as shown in Figure 4.

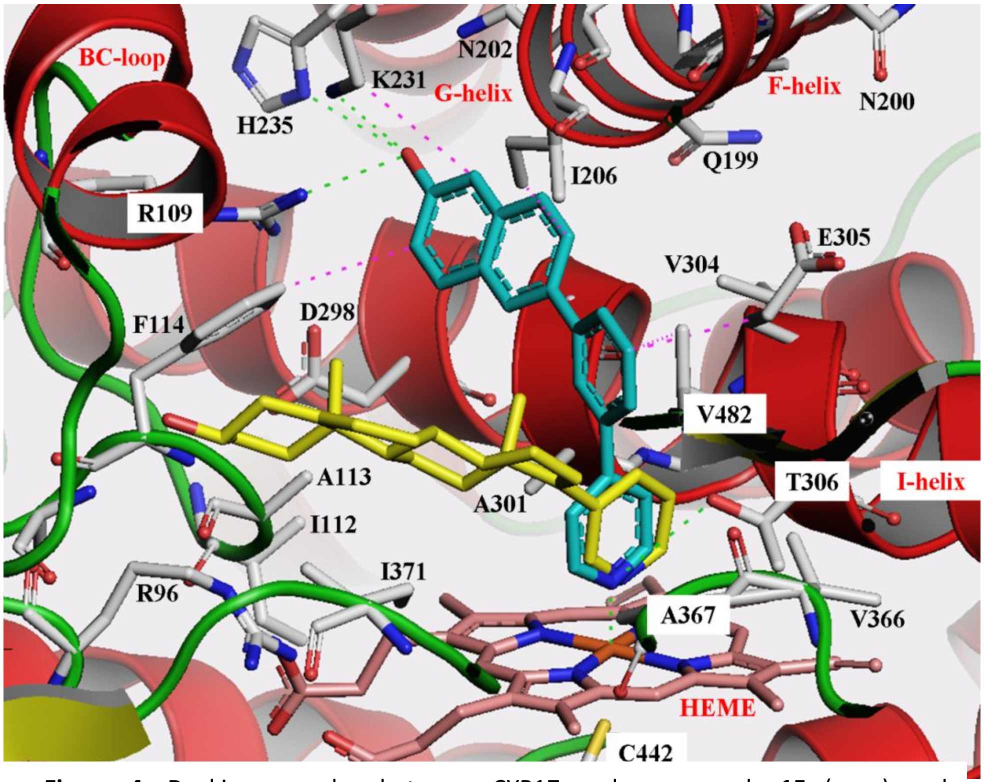


Figure 4. Docking complex between CYP17 and compounds **15** (cyan) and abiraterone (yellow). Heme, interacting residues and ribbon rendered tertiary structure of the active site are shown. Figure was generated with Pymol (http://www.pymol.org).

Besides the already described interaction between the sp^2 hybridised nitrogen and the heme iron, the key interaction for compound **15** seems to be the H-bond net of the hydroxyl group on the naphthalene with the amino acids Arg109, His235, Lys231 and Asn202 in the active site of our target enzyme. Further, hydrophobic interactions between the aromatic core and Ile206, Gly301, Ala302, Glu305, Val366 and Val482 were observed.

For abiraterone, the same binding mode as described for the substrates was found.[29,30] The lone pair of the sp^2 hybridised nitrogen pointed perpendicular towards the heme iron. The steroidal scaffold was oriented almost parallel to the heme plane in the direction of the BC-loop. This pose was stabilised by hydrophobic interactions with Ile371, Ile112, Ala113 and Phe114.[29,30] Additionally, the highly conserved Arg96 which is important for substrate binding and recognition, as shown by sitedirected mutagenesis,[31] presented interactions of the same kind with the steroidal A-ring. Another important interaction was the H-bond between the hydroxyl group in C3 position and the backbone carbonyl group of Gln98.

Discussion and Conclusion

Relying on our molecular modelling studies, the inhibitory activity of compound **15** is supposed to be increased by introducing other polar groups like an H-bond donor in 7 position of the naphthalene, which could interact with Asp298. Other possible substitutions to increase activity and perhaps also selectivity towards hepatic CYP enzymes are chlorine or fluorine at the 1 position of the naphthalene, which should improve the hydrophobic interactions with the I-helix. Also a substituent at 3 position capable of undergoing H-bonds could interact with Glu204.

The selectivity against CYP1A2 and CYP2D6 could also be enhanced through different medicinal chemistry strategies. It is described that planar, lipophilic structures[32] like compound **15** have a great affinity towards CYP1A2. This planarity could be abolished by either dearomatising one of the benzene rings, or by specific ortho-substitutions. Regarding CYP2D6, its high inhibition is not surprising, since compound **15** bears three important pharmacophores responsible for its inhibition: the aromatic nitrogen, the H-bond donor and the lipophilic core structure.[33] Since CYP2D6 has a relatively small binding site, most of the changes or substitutions made on compound **15** might increase its selectivity against this enzyme.

Summarising, we have discovered compound **15** which shows good inhibition values, a good selectivity towards the steroidogenic CYP11B1 and a modest selectivity against CYP3A4, the most relevant hepatic enzyme in xenobiotic metabolism. Compound **15** might therefore be a good candidate for further structure optimisation. Using our protein model, we made some suggestions of how to further increase activity and selectivity towards hepatic CYP enzymes.

Molecular docking calculations were performed for compound **15**. 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.

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 Ionisation) and APCI (Atmospheric Pressure Chemical Ionisation) mass spectra were determined on a TSQ quantum (Thermo Electron Corporation) instrument. EI (Electron Ionisation) mass spectra were determined on a Trace GC Ultra (Thermo Electron Corporation) instrument. All reactions in the microwave were performed in a closed vessel in a CEM Discovery^{TM} instrument equipped with an IR-sensor for temperature control. 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 and other compounds used as starting materials were commercially obtained (CombiBlocks, Chempur, Aldrich, Acros).

1,6-Dibromonaphthalene, 1b. To an ice-cooled solution of triphenylphosphine (16.40 g, 62.50 mmol) in acetonitrile (30 mL) was carefully dropped bromine (3.20 mL, 62.50 mmol). Then naphthalene-1,6-diol (5.00 g, 31.25 mmol) was added and the resulting mixture was heated for 2 hours at 80 °C. After that the solvent was evaporated under reduced pressure and the residue was heated in the microwave at 240 °C for 5 minutes. The crude was boiled in ethanol for 30 minutes, followed by hot and cold filtration. After evaporation under reduced pressure, the desired product was purified by chromatography on silica gel; yield: 6.35 g (71 %); white solid; R_f = 0.69 (hexane / EtOAc, 80:20); δ_H ($CDCl_3$, 500 MHz) 7.32-7.35 (m, 2H), 7.65 (dd, *J* = 1.2, 9.0 Hz, 1H), 7.70 (d, *J* = 8.2 Hz, 1H), 7.78 (d, *J* = 7.4 Hz, 1H), 7.99 (s, 1H), 8.10 (d, *J* = 9.0 Hz, 1H); MS (EI): m/z = 283.8, 285.8, 287.8 [M^++H].

Method A: Suzuki-Coupling. The corresponding brominated aromatic compound (1 eq) was dissolved in toluene (7 mL / mmol), a 10 % Na_2CO_3 $_{aq}$ solution (2 eq) and 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, $Pd(PPh_3)_4$ (5 mol%) was added and the resulting suspension was heated under reflux for 16 hours. After cooling and phase separation, the aqueous phase was extracted with ethyl acetate (3 x 25 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.

1-Bromo-6-(4-fluorophenyl)naphthalene, 1a. Synthesised according to Method A using compound **1b** (0.75 g, 2.62 mmol) and 4-fluorophenylboronic acid (0.45 g, 3.22 mmol); yield: 0.33 g (42 %); white solid; R_f = 0.78 (hexane / EtOAc, 80:20); MS (EI): m/z = 312.9, 316.0 [M^++H].

3-(6-(4-Fluorophenyl)naphthalen-1-yl)pyridine, 1. Synthesised according to Method A using compound **1a** (0.18 g, 0.58 mmol) and 3-pyridineboronic acid (0.15 g, 1.16 mmol); yield: 0.05 g (31 %); light-yellow oil; R_f = 0.57 (DCM / MeOH, 98:2); IR (ATR) υ (cm^{-1}) 3038 (w), 1602 (w), 1517 (m), 1501 (s), 1407 (w), 1229 (s), 1159 (m), 1100 (w), 1026 (w), 965 (w), 888 (w), 844 (m), 823 (s), 792 (s), 759 (m), 715 (s), 659 (m), 539 (m); δ_H ($CDCl_3$, 500 MHz) 7.16-7.20 (m, 2H), 7.43 (dd, *J* = 1.1, 7.0 Hz, 1H), 7.47 (dd, *J* = 4.5, 7.7 Hz, 1H), 7.59 (dd, *J* = 7.1, 8.2 Hz, 1H), 7.66-7.69 (m, 2H), 7.84-7.88 (m, 1H), 7.88-7.89 (m, 1H), 7.96 (d, *J* = 8.3 Hz, 1H), 8.07 (d, *J* = 1.8 Hz, 1H), 8.71 (d, *J* = 3.6 Hz, 1H), 8.79 (d, *J* = 1.1 Hz, 1H); δ_C ($CDCl_3$, 125 MHz) 115.7, 115.9, 123.2, 125.9, 126.0, 126.1, 127.5, 128.7, 128.9, 129.0, 130.6, 134.1, 136.2, 136.3, 136.79, 136.81, 137.3, 137.8, 148.5, 148.7, 150.6, 162.7 (d, *J* = 247.0 Hz, C-F); MS (ESI): *m/z* = 299.9 [M^++H].

4-(6-(4-Fluorophenyl)naphthalen-1-yl)pyridine, 2. Synthesised according to Method A using compound **1a** (0.10 g, 0.33 mmol) and 4-pyridineboronic acid (0.06 g, 0.43 mmol); yield: 0.09 g (94 %); light-yellow oil; R_f = 0.69 (DCM / MeOH, 95:5); IR (ATR) υ (cm^{-1}) 3054 (w), 1732 (w), 1601 (m), 1504 (s), 1432 (w), 1409 (w), 1264 (w), 1231 (s), 1159 (m), 879 (w), 823 (s), 794 (s), 760 (s), 654 (m), 605 (w), 518 (w); δ_H ($CDCl_3$, 500 MHz) 7.16-7.20 (m, 2H), 7.43 (dd, *J* = 1.0, 7.1 Hz, 1H), 7.56-7.60 (m, 3H), 7.66-7.70 (m, 3H), 7.89 (d, *J* = 8.8 Hz, 1H), 7.98 (d, *J* = 8.3 Hz, 1H), 8.08 (d, *J* = 1.8 Hz, 1H), 8.84 (d, *J* = 3.3 Hz, 2H); δ_C ($CDCl_3$, 125 MHz) 115.8, 115.9, 125.5, 125.6, 125.9, 126.2, 126.3, 127.0, 128.9, 129.0, 129.4, 129.6, 134.0, 136.5, 136.6, 138.0, 149.5, 150.0, 162.7 (d, *J* = 247.1 Hz, C-F); MS (ESI): *m/z* = 300.0 [M^++H].

1-Bromo-6-(4-fluorophenyl)-2-methoxynaphthalene, 3a. Synthesised according to Method A using compound **3b** (1.00 g, 3.17 mmol) and 4-fluorophenylboronic acid (0.45 g, 3.17 mmol); yield: 0.84 g (84 %); white solid; R_f = 0.57 (hexane / EtOAc, 80:20); δ_H ($CDCl_3$, 500 MHz) 4.05 (s, 3H), 7.15-7.19 (m, 2H), 7.31 (d, *J* = 9.0 Hz, 1H), 7.66 (dd, *J* = 5.3, 8.8 Hz, 2H), 7.77 (dd, *J* = 1.9, 8.9 Hz, 1H), 7.86 (d, *J* = 9.0 Hz, 1H), 7.93 (d, *J* = 1.7 Hz, 1H), 8.28 (d, *J* = 8.9 Hz, 1H); δ_C ($CDCl_3$, 125 MHz) 57.1 (CH_3), 108.6, 114.1, 115.7, 115.9, 125.6, 126.9, 127.2, 128.76, 128.83, 129.1, 130.0, 132.3, 136.1, 136.6, 136.7, 152.9, 162.6 (d, *J* = 246.7 Hz, C-F); MS (EI): *m/z* = 329.9, 332.9 [M^++H].

3-(6-(4-Fluorophenyl)-2-methoxynaphthalen-1-yl)pyridine, 3. Synthesised according to Method A using compound **3a** (0.30 g, 0.91 mmol) and 3-pyridineboronic acid (0.14 g, 1.09 mmol); yield: 0.20 g (66 %); white solid: mp 147 °C; R_f = 0.57 (DCM / MeOH, 95:5); IR (ATR) υ

(cm^{-1}) 2926 (m), 1599 (m), 1516 (m), 1496 (m), 1460 (w), 1344 (w), 1257 (s), 1164 (w), 1134 (w), 1067 (s), 1025 (w), 823 (s), 804 (s), 750 (w), 717 (m), 626 (w), 522 (w); δ_H ($CDCl_3$, 500 MHz) 3.87 (s, 3H), 7.14-7.17 (m, 2H), 7.41 (d, *J* = 9.1 Hz, 1H), 7.47 (dd, *J* = 4.9, 7.7 Hz, 1H), 7.53 (d, *J* = 8.9 Hz, 1H), 7.59 (dd, *J* = 1.9, 8.9 Hz, 1H), 7.65 (dd, *J* = 5.3, 8.8 Hz, 2H), 7.76 (td, *J* = 1.9, 7.8 Hz, 1H), 7.97-7.99 (m, 2H), 8.66 (d, *J* = 1.5 Hz, 1H), 8.68 (dd, *J* = 1.5, 4.9 Hz, 1H); MS (APCI): *m/z* = 320.3 [M^++H].

2-Bromo-6-(4-fluorophenyl)naphthalene, 5a. Synthesised according to Method A using 2,6-dibromonaphthalene (0.70 g, 2.47 mmol) and 4-fluorophenylboronic acid (0.35 g, 2.47 mmol); yield: 0.23 g (37 %); white solid; R_f = 0.80 (hexane / EtOAc, 80:20); δ_H ($CDCl_3$, 500 MHz) 7.16-7.19 (m, 2H), 7.58 (dd, *J* = 2.0, 8.7 Hz, 1H), 7.66 (dd, *J* = 5.3, 8.8 Hz, 2H), 7.71 (dd, *J* = 1.8, 8.6 Hz, 1H), 7.75 (d, *J* = 8.7 Hz, 1H), 7.82 (d, *J* = 8.6 Hz, 1H), 7.95 (d, *J* = 1.0 Hz, 1H), 8.03 (d, *J* = 1.7 Hz, 1H); δ_C ($CDCl_3$, 125 MHz) 115.7, 115.9, 119.9, 125.5, 126.5, 127.6, 128.88, 128.94, 129.70, 129.74, 129.8, 132.1, 133.6, 136.78, 136.80, 138.1, 162.7 (d, *J* = 247.0 Hz, C-F); MS (EI): m/z = 299.8, 302.9 [M^++H].

3-(6-(4-Fluorophenyl)naphthalen-2-yl)pyridine, 5. Synthesised according to Method A using compound **5a** (0.15 g, 0.50 mmol) and 3-pyridineboronic acid (0.08 g, 0.65 mmol); yield: 0.07 g (47 %); light-yellow solid: mp 126 °C; R_f = 0.72 (DCM / MeOH, 95:5); IR (ATR) υ (cm^{-1}) 2961 (m), 1728 (s), 1605 (m), 1516 (m), 1487 (m), 1377 (w), 1286 (s), 1240 (s), 1122 (s), 1072 (s), 981 (w), 896 (m), 812 (s), 744 (m), 706 (m), 666 (m), 544 (w), 527 (m); δ_H ($CDCl_3$, 500 MHz) 7.17-7.21 (m, 2H), 7.46 (dd, *J* = 4.9, 7.7 Hz, 1H), 7.53 (dd, *J* = 3.3, 5.7 Hz, 1H), 7.69 (dd, *J* = 5.3, 8.8 Hz, 2H), 7.73 (dd, *J* = 3.3, 5.7 Hz, 1H), 7.75 (dd, *J* = 1.9, 4.7 Hz, 1H), 7.77 (dd, *J* = 1.9, 4.7 Hz, 1H), 7.98-8.02 (m, 2H), 8.03 (d, *J* = 1.2 Hz, 1H), 8.05-8.08 (m, 2H), 8.65 (d, *J* = 3.7 Hz, 1H), 9.01 (s, 1H); δ_C ($CDCl_3$, 125 MHz) 115.7, 115.9, 123.8, 125.4, 125.5, 126.0, 126.2, 128.84, 128.89, 128.92, 128.99, 132.7, 133.2, 134.97, 135.02, 136.94, 136.97, 138.3, 147.92, 147.94, 162.7 (d, *J* = 247.2 Hz, C-F); MS (ESI): *m/z* = 300.2 [M^++H].

6-(4-Fluorophenyl)-1-naphthoic acid, 6a. Synthesised according to Method A using compound **6b** (0.50 g, 2.11 mmol) and 4-fluorophenylboronic acid (0.39 g, 2.74 mmol); yield: 0.45 g (85 %); white solid; R_f = 0.44 (DCM / MeOH, 98:2); δ_H ($CDCl_3$, 500 MHz) 5.16 (s, 2H), 7.16-7.20 (m, 2H), 7.46-7.53 (m, 2H), 7.68 (dd, *J* = 5.3, 8.7 Hz, 2H), 7.75 (dd, *J* = 1.9, 8.7 Hz, 1H), 7.86 (d, *J* = 8.0 Hz, 1H), 8.02 (d, *J* = 1.7 Hz, 1H), 8.19 (d, *J* = 8.8 Hz, 1H); δ_C ($CDCl_3$, 125 MHz) 63.7 (CH_2), 115.7, 115.8, 124.4, 125.4, 125.8, 126.0, 126.3, 128.8, 128.9, 130.3, 134.0, 136.2, 136.91, 136.93, 137.5, 162.6 (d, *J* = 246.8 Hz, C-F); MS (EI): m/z = 252.0 [M^++H].

6-(4-Fluorophenyl)-2-methoxy-1-naphthaldehyde, 7b. Synthesised according to Method A using compound **7c** (1.00 g, 3.77 mmol) and 4-fluorophenylboronic acid (0.54 g, 3.77 mmol); yield: 0.63 g (60 %); white solid; R_f = 0.32 (hexane / EtOAc, 80:20); δ_H ($CDCl_3$, 500 MHz) 4.08 (s, 3H), 7.15-7.19 (m, 2H), 7.34 (d, *J* = 9.2 Hz, 1H), 7.66 (dd, *J* = 5.3, 8.8 Hz, 2H), 7.84 (dd, *J* = 2.1, 9.0

Hz, 1H), 7.92 (d, *J* = 2.0 Hz, 1H), 8.11 (d, *J* = 9.1 Hz, 1H), 9.34 (d, *J* = 9.0 Hz, 1H); MS (APCI): m/z = 322.2 [M^++H].

(6-(4-Fluorophenyl)naphthalen-2-yl)methanol, 8a. Synthesised according to Method A using compound **8b** (0.40 g, 1.69 mmol) and 4-fluorophenylboronic acid (0.29 g, 2.03 mmol); yield: 0.36 g (84 %); white solid; R_f = 0.52 (DCM / MeOH, 98:2); δ_H ($CDCl_3$, 500 MHz) 4.88 (s, 2H), 7.16-7.19 (m, 2H), 7.51 (dd, *J* = 1.4, 8.4 Hz, 1H), 7.68 (dd, *J* = 5.3, 8.7 Hz, 2H), 7.70 (dd, *J* = 1.8, 8.5 Hz, 1H), 7.83 (s, 1H), 7.89 (dd, *J* = 5.2, 8.4 Hz, 2H), 7.98 (s, 1H); δ_C ($CDCl_3$, 125 MHz) 65.4 (CH_2), 115.6, 115.8, 125.2, 125.4, 125.7, 128.5, 128.6, 128.86, 128.93, 132.4, 133.1, 137.10, 137.12, 137.6, 138.5, 162.5 (d, *J* = 246.6 Hz, C-F); MS (APCI): m/z = 235.1, 236.0, 237.0 [M^++H].

3-(4-Fluorophenyl)-1-naphthoic acid, 9a. Synthesised according to Method A using compound **9b** (0.50 g, 2.11 mmol) and 4-fluorophenylboronic acid (0.39 g, 2.74 mmol); yield: 0.42 g (78 %); white solid; R_f = 0.50 (DCM / MeOH, 98:2); δ_H ($CDCl_3$, 500 MHz) 5.22 (s, 2H), 7.16-7.19 (m, 2H), 7.52-7.58 (m, 2H), 7.69 (dd, *J* = 5.3, 8.8 Hz, 2H), 7.76-7.77 (m, 1H), 7.92-7.94 (m, 1H), 7.96 (s, 1H), 8.11-8.13 (m, 1H); δ_C ($CDCl_3$, 125 MHz) 63.7 (CH_2), 103.2, 115.7, 115.8, 123.5, 126.0, 126.42, 126.44, 128.86, 128.92, 130.3, 134.1, 137.0, 162.6 (d, *J* = 246.9 Hz, C-F); MS (EI): m/z = 252.0 [M^++H].

1-Bromo-6-(4-fluoro-2-methylphenyl)naphthalene, 10a. Synthesised according to Method A using compound **1b** (0.75 g, 2.62 mmol) and 2-methyl-4-fluorophenylboronic acid (0.50 g, 3.28 mmol); yield: 0.26 g (31 %); white solid; R_f = 0.85 (hexane / EtOAc, 80:20); δ_H ($CDCl_3$, 500 MHz) 2.29 (s, 3H), 6.98 (dt, *J* = 2.3, 8.4 Hz, 1H), 7.03 (dd, *J* = 2.5, 9.7 Hz, 1H), 7.27 (dd, *J* = 5.9, 8.6 Hz, 1H), 7.34-7.37 (m, 1H), 7.54 (dd, *J* = 1.7, 8.7 Hz, 1H), 7.74 (d, *J* = 1.5 Hz, 1H), 7.80 (dd, *J* = 1.0, 7.6 Hz, 1H), 7.82 (d, *J* = 8.3 Hz, 1H), 8.28 (d, *J* = 8.7 Hz, 1H); δ_C ($CDCl_3$, 125 MHz) 20.6 (CH_3), 112.6, 112.8, 116.8, 116.9, 117.0, 122.7, 126.5, 126.6, 126.8, 127.0, 127.3, 127.7, 127.9, 128.19, 128.23, 128.7, 129.1, 129.4, 129.9, 130.1, 130.2, 130.6, 130.9, 131.3, 131.4, 131.6, 134.5, 137.09, 137.12, 137.9, 138.0, 139.5, 162.2 (d, *J* = 246.0 Hz, C-F); MS (ESI): *m/z = not ionisable* [M^++H].

3-(6-(4-Fluoro-2-methylphenyl)naphthalen-1-yl)pyridine, 10. Synthesised according to Method A using compound **10a** (0.15 g, 0.48 mmol) and 3-pyridineboronic acid (0.09 g, 0.71 mmol); yield: 0.05 g (31 %); colourless oil; R_f = 0.51 (DCM / MeOH, 98:2); IR (ATR) υ (cm^{-1}) 3049 (w), 1585 (w), 1493 (s), 1408 (w), 1270 (m), 1222 (s), 1186 (w), 1152 (m), 1025 (w), 965 (m), 866 (m), 836 (w), 810 (s), 794 (s), 761 (s), 717 (s), 657 (m), 583 (m); δ_H ($CDCl_3$, 500 MHz) 2.30 (s, 3H), 6.98 (dt, *J* = 2.7, 8.4 Hz, 1H), 7.02 (dd, *J* = 2.6, 9.8 Hz, 1H), 7.28 (dd, *J* = 6.0, 8.4 Hz, 1H), 7.42 (dd, *J* = 1.8, 8.7 Hz, 1H), 7.45 (dd, *J* = 0.9, 7.1 Hz, 1H), 7.46 (dd, *J* = 4.9, 7.8 Hz, 1H), 7.60 (dd, *J* = 7.1, 8.2 Hz, 1H), 7.83 (d, *J* = 1.6 Hz, 1H), 7.85 (d, *J* = 8.8 Hz, 1H), 7.87 (td, *J* = 1.8, 7.8 Hz, 1H), 7.93 (d, *J* = 8.3 Hz, 1H), 8.71 (dd, *J* = 1.6, 4.9 Hz, 1H), 8.80 (d, *J* = 2.1 Hz, 1H); δ_C ($CDCl_3$, 125 MHz) 20.6, 20.7, 112.0, 112.6, 112.7, 116.8, 117.0, 123.2, 125.1, 125.9, 127.4, 128.4, 128.5, 128.6, 130.4, 130.8, 131.3, 131.4, 133.7, 136.3, 137.3, 137.4, 138.0, 138.9, 148.6, 150.6, 162.2 (d, *J* = 245.9 Hz, C-F); MS (ESI): *m/z* = 313.9 [M^++H].

3-(5-Bromo-6-methoxynaphthalen-2-yl)pyridine, 11a. Synthesised according to Method A using compound **3b** (0.75 g, 2.41 mmol) and 3-pyridineboronic acid (0.36 g, 2.89 mmol); yield: 0.19 g (25 %); white solid; R_f = 0.22 (DCM / MeOH, 98:2); δ_H ($CDCl_3$, 500 MHz) 3.98 (s, 3H), 7.26 (d, *J* = 9.0 Hz, 1H), 7.34 (ddd, *J* = 0.7, 4.8, 7.9 Hz, 1H), 7.72 (dd, *J* = 1.9, 8.8 Hz, 1H), 7.82 (d, *J* = 9.0 Hz, 1H), 7.90-7.92 (m, 2H), 8.25 (d, *J* = 8.8 Hz, 1H), 8.56 (dd, *J* = 1.5, 4.8 Hz, 1H), 8.90 (d, *J* = 1.9 Hz, 1H); δ_C ($CDCl_3$, 125 MHz) 57.1, 108.5, 114.2, 123.6, 126.1, 126.8, 127.2, 129.3, 129.9, 132.7, 133.6, 134.4, 136.0, 148.4, 148.6, 154.2, 190.7; MS (EI): *m/z* = 314.9, 316.0 [M^++H].

3-(5-(4-Fluorophenyl)-6-methoxynaphthalen-2-yl)pyridine, 11. Synthesised according to Method A using compound **11a** (0.10 g, 0.32 mmol) and 4-fluorophenylboronic acid (0.07 g, 0.48 mmol); yield: 0.08 g (77 %); white solid; R_f = 0.23 (DCM / MeOH, 98:2); IR (ATR) υ (cm^{-1}) 2840 (w), 1735 (w), 1599 (w), 1512 (m), 1488 (m), 1463 (w), 1281 (w), 1256 (s), 1214 (m), 1159 (w), 1132 (w), 1068 (s), 1018 (w), 914 (w), 900 (w), 833 (m), 808 (s), 758 (w), 715 (s), 689 (w), 625 (w), 556 (m), 535 (m); δ_H ($CDCl_3$, 500 MHz) 3.87 (s, 3H), 7.19-7.23 (m, 2H), 7.36 (dd, *J* = 5.5, 8.7 Hz, 2H), 7.42 (dd, *J* = 4.9, 7.8 Hz, 1H), 7.43 (d, *J* = 9.1 Hz, 1H), 7.56-7.60 (m, 2H), 7.96-8.00 (m, 2H), 8.04 (s, 1H), 8.63 (dd, *J* = 1.3, 4.8 Hz, 1H), 8.96 (d, *J* = 1.8 Hz, 1H); δ_C ($CDCl_3$, 125 MHz) 56.6, 114.3, 115.2, 115.3, 123.7, 124.1, 125.5, 126.1, 126.2, 129.1, 129.7, 131.79, 131.81, 132.48, 132.54, 132.6, 133.2, 134.6, 136.6, 148.2, 148.3, 154.3, 162.1 (d, *J* = 245.9 Hz, C-F); MS (ESI): *m/z* = 329.9 [M^++H].

2-(3-Bromophenyl)-6-methoxynaphthalene, 12a. Synthesised according to Method A using 1,3-dibromobenzene (0.93 mL, 7.43 mmol) and 3-methoxyphenylboronic acid (1.50 g, 7.43 mmol); yield: 1.82 g (78 %); white solid; R_f = 0.68 (DCM / MeOH, 98:2); δ_H ($CDCl_3$, 500 MHz) 4.88 (s, 2H), 7.17 (d, *J* = 2.5 Hz, 1H), 7.19 (dd, *J* = 2.6, 8.9 Hz, 1H), 7.32-7.35 (m, 2H), 7.47-7.49 (m, 1H), 7.61-7.63 (m, 1H), 7.66 (dd, *J* = 1.8, 8.4 Hz, 1H), 7.79-7.82 (m, 2H), 7.84-7.85 (m, 1H), 7.95 (d, *J* = 1.7, 1H); δ_C ($CDCl_3$, 125 MHz) 55.4 (CH_3), 105.6, 119.4, 123.0, 125.7, 125.78, 125.82, 127.4, 128.5, 129.1, 129.8, 129.9, 130.2, 130.3, 131.1, 134.1, 134.8, 143.4, 158.0; MS (EI): m/z = 312.9, 316.0 [M^++H].

3-(3-(6-Methoxynaphthalen-2-yl)phenyl)pyridine, 12. Synthesised according to Method A using compound **12a** (0.30 g, 0.96 mmol) and 3-pyridineboronic acid (0.15 g, 1.15 mmol); yield: 0.22 g (74 %); light-yellow solid: mp 157 °C; R_f = 0.71 (DCM / MeOH, 95:5); IR (ATR) υ (cm^{-1}) 2925 (w), 1605 (w), 1483 (w), 1385 (w), 1268 (w), 1244 (m), 1205 (s), 1165 (m), 1026 (m), 858 (s), 819 (s), 792 (s), 714 (s); δ_H ($CDCl_3$, 500 MHz) 3.95 (s, 3H), 7.19-7.21 (m, 2H), 7.42 (dd, *J* = 4.9, 7.7 Hz, 1H), 7.59 (m, 2H), 7.73-7.77 (m, 2H), 7.82 (d, *J* = 8.7 Hz, 1H), 7.85 (d, *J* = 8.5 Hz, 1H), 7.90 (s, 1H), 7.99 (td, *J* = 1.6, 7.9 Hz, 1H), 8.03 (s, 1H), 8.63 (d, *J* = 4.1 Hz, 1H), 8.95 (d, *J* = 1.3 Hz, 1H);

δ_C ($CDCl_3$, 125 MHz) 55.4, 105.6, 119.3, 123.7, 125.80, 125.84, 125.9, 126.1, 127.1, 127.4, 129.1, 129.6, 129.7, 134.0, 134.7, 135.8, 142.2, 148.2, 148.3, 157.9; MS (ESI): *m/z* = 312.2 [M^++H].

4-(3-(6-Methoxynaphthalen-2-yl)phenyl)pyridine, 13. Synthesised according to Method A using compound **12a** (0.40 g, 1.28 mmol) and 4-fluorophenylboronic acid (0.19 g, 1.53 mmol); yield: 0.26 g (67 %); white solid; R_f = 0.54 (DCM / MeOH, 95:5); IR (ATR) υ (cm^{-1}) 3058 (w), 1593 (s), 1546 (w), 1489 (m), 1386 (w), 1268 (w), 1244 (m), 1205 (s), 1164 (w), 1025 (m), 895 (w), 857 (s), 828 (w), 816 (s), 795 (s), 707 (w), 616 (m), 573 (w), 537 (w), 510 (w); δ_H ($CDCl_3$, 500 MHz) 3.95 (s, 3H), 7.18 (d, *J* = 2.5 Hz, 1H), 7.20 (dd, *J* = 2.5, 8.9 Hz, 1H), 7.58-7.63 (m, 4H), 7.76 (dd, *J* = 1.8, 8.5 Hz, 1H), 7.77 (td, *J* = 1.5, 7.4 Hz, 1H), 7.81 (d, *J* = 8.9 Hz, 1H), 7.84 (d, *J* = 8.5 Hz, 1H), 7.93-7.94 (m, 1H), 8.02 (d, *J* = 1.5 Hz, 1H), 8.69-8.70 (m, 2H); δ_C ($CDCl_3$, 125 MHz) 55.3, 105.5, 119.3, 121.6, 121.7, 125.6, 125.7, 125.8, 125.9, 126.9, 127.4, 127.8, 129.05, 129.07, 129.1, 129.5, 129.7, 133.9, 135.7, 138.7, 142.1, 148.3, 150.18, 150.24, 157.9; MS (ESI): *m/z* = 312.1 [M^++H].

3-(6-Methoxynaphthalen-2-yl)benzaldehyde, 16b. Synthesised according to Method A using 3-bromobenzaldehyde (1.50 g, 7.58 mmol) and 3-methoxyphenylboronic acid (1.00 mL, 8.33 mmol); yield: 1.81 g (91 %); white solid; R_f = 0.51 (hexane / EtOAc, 80:20); MS (EI): m/z = 262.0 [M^++H].

4-(6-(*tert*-Butyldimethylsilyloxy)naphthalen-2-yl)benzaldehyde, 17c. Synthesised accord-ing to Method A using compound **17d** (6.18 g, 18.30 mmol) and 4-formylphenylboronic acid (4.13 g, 27.50 mmol). Then the desired product was taken to the next step without further purification; yield: 6.07 g (91 %); R_f = 0.43 (hexane / EtOAc, 90:10).

1,6-Dibromo-2-methoxynaphthalene, 3b. To a solution of 2-bromo-6-methoxynaphthalene (3.65 g, 15.24 mmol) in THF (30 mL) was added N-bromosuccinimide (3.01 g, 16.76 mmol). Then the solution was heated to reflux for 2 hours. After cooling to ambient temperature, it was diluted with water (50 mL) and extracted with ethyl acetate (3 x 25 mL). The combined organic phases were washed with 1 N HCl, brine, dried over Na_2SO_4 and evaporated under reduced pressure. Then the desired product was taken to the next step without further purification; yield: 4.82 g (100 %); white solid; R_f = 0.48 (hexane / EtOAc, 80:20); δ_H ($CDCl_3$, 500 MHz) 4.02 (s, 3H), 7.27 (d, *J* = 9.5 Hz, 1H), 7.60 (d, *J* = 9.1 Hz, 1H), 7.71 (d, *J* = 8.8 Hz, 1H), 7.92 (s, 1H), 8.08 (d, *J* = 9.1 Hz, 1H); δ_C ($CDCl_3$, 125 MHz) 57.0 (CH_3), 108.7, 114.5, 118.1, 128.0, 129.8, 130.6, 130.9, 131.7, 154.0, 171.0; MS (ESI): m/z = *not ionisable* [M^++H].

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.

6-(4-Fluorophenyl)-1-(pyridin-3-yl)naphthalen-2-ol, 4. Synthesised according to Method B using **3** (0.14 g, 0.43 mmol) and BBr_3 (2.20 mL, 2.20 mmol); yield: 0.10 g (75 %); light-yellow solid: mp 234 °C; R_f = 0.42 (DCM / MeOH, 95:5); IR (ATR) υ (cm^{-1}) 2922 (w), 1595 (w), 1498 (s), 1350 (m), 1324 (w), 1291 (m), 1257 (w), 1228 (m), 1188 (w), 1159 (w), 1052 (w), 943 (m), 846 (w), 816 (s), 769 (w), 710 (s), 662 (w), 638 (m), 520 (m); δ_H ($CDCl_3$ + CD_3OD, 500 MHz) 7.01-7.04 (m, 2H), 7.16 (d, *J* = 8.9 Hz, 1H), 7.34 (d, *J* = 8.8 Hz, 1H), 7.42-7.46 (m, 2H), 7.51-7.53 (m, 2H), 7.72 (d, *J* = 8.9 Hz, 1H), 7.78 (dd, *J* = 1.6, 7.8 Hz, 1H), 7.84 (s, 1H), 8.48 (d, *J* = 4.6 Hz, 1H), 8.51 (s, 1H).; δ_C ($CDCl_3$ + CD_3OD, 125 MHz) 115.8, 116.0, 117.59, 117.60, 118.8, 124.1, 124.7, 126.1, 126.4, 128.87, 128.94, 129.1, 130.5, 133.0, 133.4, 135.2, 137.29, 137.32, 140.2, 147.4, 151.2, 152.4, 162.7 (d, *J* = 245.9 Hz, C-F); MS (ESI): *m/z* = 314.0 [M^++H].

6-(3-(Pyridin-3-yl)phenyl)naphthalen-2-ol, 14. Synthesised according to Method B using **12** (0.09 g, 0.29 mmol) and BBr_3 (1.00 mL, 1.00 mmol); yield: 0.06 g (67 %); light-yellow solid: mp 223 °C; R_f = 0.47 (DCM / MeOH, 95:5); IR (ATR) υ (cm^{-1}) 2962 (w), 1626 (w), 1596 (m), 1482 (w), 1428 (m), 1396 (w), 1260 (m), 1236 (m), 1199 (m), 1104 (s), 1020 (s), 848 (w), 783 (s), 703 (s), 674 (w); δ_H ($CDCl_3$ + CD_3OD 500 MHz) 7.11 (dd, *J* = 2.4, 8.8 Hz, 1H), 7.14 (d, *J* = 2.3 Hz, 1H), 7.45-7.48 (m, 1H), 7.50-7.52 (m, 2H), 7.52-7.56 (q, *J* = 7.6 Hz, 1H), 7.65-7.67 (dd, *J* = 1.8, 8.6 Hz, 1H), 7.70-7.72 (m, 2H), 7.74 (d, *J* = 8.8 Hz, 1H), 7.83-7.84 (m, 1H), 7.95 (s, 1H), 8.04 (d, *J* = 7.9 Hz, 1H), 8.56 (s, 1H), 8.88 (s, 1H); δ_C ($CDCl_3$ + CD_3OD, 125 MHz) 109.0, 113.1, 118.8, 125.6, 125.7, 125.8, 126.0, 127.0, 127.3, 128.6, 129.6, 129.8, 134.3, 135.0, 149.9, 155.0, 156.9, 160.2; MS (ESI): *m/z* = 298.3 [M^++H].

6-(3-(Pyridin-4-yl)phenyl)naphthalen-2-ol, 15. Synthesised according to Method B using **13** (0.20 g, 0.64 mmol) and BBr_3 (2.60 mL, 2.60 mmol); yield: 0.16 g (86 %); light-yellow solid; R_f = 0.38 (DCM / MeOH, 95:5); IR (ATR) υ (cm^{-1}) 3300-2450 (w), 1625 (w), 1598 (m), 1546 (w), 1494 (w), 1450 (w), 1398 (w), 1240 (m), 1218 (w), 1203 (m), 1126 (w), 1069 (w), 1007 (m), 981 (w), 860 (m), 835 (m), 812 (w), 785 (s), 697 (m), 624 (m), 571 (w); δ_H ($(CD_3)_2SO$, 500 MHz) 7.13 (dd, *J* = 2.4, 8.8 Hz, 1H), 7.17 (d, *J* = 2.2 Hz, 1H), 7.63-7.65 (m, 1H), 7.77-7.81 (m, 2H), 7.84-7.88 (m, 5H), 8.13-8.14 (m, 1H), 8.22 (s, 1H), 8.66-8.68 (m, 2H), 9.83 (s, 1H); δ_C ($(CD_3)_2SO$, 125 MHz) 108.4, 119.0, 121.4, 125.0, 125.3, 125.4, 126.7, 127.4, 127.9, 129.78, 129.81, 133.7, 134.0, 137.9, 141.2, 147.0, 150.1, 155.6; MS (ESI): *m/z* = 298.2 [M^++H].

5-Bromobenzo[de]isochromene-1,3-dione, 6d. Synthesised according to Mitchell et al.[34] To a solution of benzo[de]isochromene-1,3-dione (20.00 g, 100.92 mmol) in sulfuric acid (400 mL) was added silver sulfate (16.00 g, 51.32 mmol) and then bromine (6.41 mL, 125.15 mmol) was carefully dropped. Then the resulting mixture was heated at 65 °C for 6 hours. After cooling to ambient temperature, the reaction mixture was filtered and then carefully poured into ice water (3.2 L). The precipitate was filtrated and washed first with water and then with ethanol. A further purification was not necessary; yield: 27.26 g (100 %); white-off solid; R_f = 0.72 (DCM / MeOH, 95:5); MS (EI): m/z = 275.8, 278.9 [M^++H].

6-Bromo-1-naphthoic acid, 6c. Synthesised according to Leuck et al.[35] To a solution of **6d** (10.10 g, 36.45 mmol) and sodium hydroxide in water (240 mL) was added a solution of yellow mercury oxide (11.00 g, 50.86 mmol) in water (30 mL) and acetic acid (10 mL). The resulting mixture was heated under reflux for 4 days. Then 5 N hydrochloric acid (200 mL) was added and the mixture heated to reflux for 4 hours. After that, the crude was filtered at 0 °C. After fractional recrystallisation from acetic acid, the more soluble **6c** remained in the mother liquor; yield: 2.10 g (23 %); white-off solid; R_f = 0.49 (hexane / EtOAc, 80:20); δ_H ($CDCl_3$, 500 MHz) 7.42-7.47 (m, 2H), 7.51-7.52 (m, 1H), 7.82 (d, *J* = 8.1 Hz, 1H), 7.96 (d, *J* = 8.2 Hz, 1H), 8.16 (d, *J* = 7.3 Hz, 1H), 8.86 (d, *J* = 8.6 Hz, 1H); δ_C ($CDCl_3$, 125 MHz) 124.6, 126.0, 126.2, 127.5, 127.7, 128.6, 130.6, 133.5, 134.0; MS (EI): m/z = 249.8, 252.9 [M^++H].

3-Bromo-1-naphthoic acid, 9c. Synthesised according to Leuck et al.[35] To a solution of **6d** (10.10 g, 36.45 mmol) and sodium hydroxide in water (240 mL) was added a solution of yellow mercury oxide (11.00 g, 50.86 mmol) in water (30 mL) and acetic acid (10 mL). The resulting mixture was heated under reflux for 4 days. Then 5 N hydrochloric acid (200 mL) was added and the mixture heated to reflux for 4 hours. After that, the crude was filtered at 0 °C. After fractional recrystallisation from acetic acid, the less soluble **9c** came in the early fractions; yield: 4.90 g (54 %); white-off solid; R_f = 0.39 (hexane / EtOAc, 80:20); δ_H ($CDCl_3$, 500 MHz) 7.51-7.54 (m, 1H), 7.59-7.62 (m, 1H), 7.76 (d, *J* = 8.2 Hz, 1H), 8.18 (d, *J* = 2.0 Hz, 1H), 8.40 (d, *J* = 2.1 Hz, 1H), 8.94 (d, J = 8.7 Hz, 1H); δ_C ($CDCl_3$, 125 MHz) 118.2, 126.0, 127.4, 127.8, 128.5, 130.1, 134.6, 136.2; MS (EI): m/z = 249.8, 252.9 [M^++H].

Method C: Reduction with $LiAlH_4$. To an ice-cooled solution of the corresponding carboxylic acid (1 eq) in THF or diethylether (5 mL / mmol) was carefully dropped $LiAlH_4$ (10 eq) in diethylether (5 mL / mmol). Then the resulting mixture was heated to reflux for 1 hour. After cooling to ambient temperature, water (25 mL) was added, and after phase separation the aqueous phase 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.

(6-Bromonaphthalen-1-yl)methanol, 6b. Synthesised according to Method C using **6c** (2.50 g, 9.96 mmol), $LiAlH_4$ (3.90 g, 99.55 mmol) and additional 25 mL THF; yield: 2.19 g (87 %); white-off solid; R_f = 0.44 (hexane / EtOAc, 80:20); δ_H ($CDCl_3$, 500 MHz) 5.09 (s, 2H), 7.44-7.47 (m, 1H), 7.51 (d, *J* = 6.9 Hz, 1H), 7.60 (dd, *J* = 2.1, 9.0 Hz, 1H), 7.70 (d, *J* = 8.1 Hz, 1H), 7.98 (d, *J* = 9.0 Hz, 1H), 8.02 (d, *J* = 2.0 Hz, 1H); MS (EI): m/z = 235.9, 238.9 [M^++H].

(6-Bromonaphthalen-2-yl)methanol, 8b. Synthesised according to Method C using 6-bromo-2-naphthoic acid (3.00 g, 11.95 mmol) and $LiAlH_4$ (4.68 g, 119.48 mmol); yield: 2.78 g (90 %); white solid; R_f = 0.19 (hexane / EtOAc, 80:20); δ_H ($CDCl_3$, 500 MHz) 4.85 (s, 2H), 7.50 (dd, *J* = 1.6, 8.5 Hz, 1H), 7.55 (dd, *J* = 2.0, 8.8 Hz, 1H), 7.70 (d, *J* = 8.8 Hz, 1H), 7.75 (d, *J* = 8.5 Hz, 1H), 7.78 (s, 1H), 8.00 (d, *J* = 1.6, 1H); δ_C ($CDCl_3$, 125 MHz) 65.2, 65.5, 119.8, 125.1, 125.3, 125.4, 125.9, 126.15, 126.18, 127.4, 127.7, 127.9, 128.3, 129.5, 129.6, 129.8, 131.8, 134.0, 138.9; MS (EI): m/z = 235.9, 238.9 [M^++H].

(3-Bromonaphthalen-1-yl)methanol, 9b. Synthesised according to Method C using **6c** (2.50 g, 9.96 mmol), $LiAlH_4$ (3.90 g, 99.55 mmol) and additional 25 mL THF; yield: 2.46 g (98 %); white-off solid; R_f = 0.34 (hexane / EtOAc, 80:20); δ_H ($CDCl_3$, 500 MHz) 5.14 (s, 2H), 7.52-7.56 (m, 2H), 7.65 (d, *J* = 1.9 Hz, 1H), 7.78 (dd, *J* = 1.9, 7.6 Hz, 1H), 7.97 (s, 1H), 8.03 (d, *J* = 7.9 Hz, 1H); δ_C ($CDCl_3$, 125 MHz) 62.8 (CH_2), 119.4, 123.6, 126.6, 126.9, 127.8, 128.1, 129.5, 130.1, 134.9, 138.4; MS (EI): m/z = 235.9, 238.9 [M^++H].

Method D: CDI reaction. To a solution of the corresponding alcohol (1 eq) in NMP or acetonitrile (10 mL / mmol) was added CDI (5 eq) and imidazole (2 eq). Then the solution was heated to reflux for 16 hours. After cooling to ambient temperature, it was diluted with water (50 mL) and 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.

1-((6-(4-Fluorophenyl)naphthalen-1-yl)methyl)-1*H*-imidazole, 6. Synthesised according to Method D using **6a** (0.15 g, 0.60 mmol), CDI (0.39 g, 2.38 mmol) and imidazole (0.04 g, 0.60 mmol); yield: 0.12 g (67 %); white solid; R_f = 0.39 (DCM / MeOH, 95:5); IR (ATR) υ (cm^{-1}) 3047 (w), 1604 (w), 1502 (s), 1287 (w), 1226 (s), 1159 (m), 1107 (w), 1075 (w), 906 (w), 817 (s), 759(m), 736 (m), 662 (m), 508 (m); δ_H ($CDCl_3$, 500 MHz) 5.61 (s, 2H), 6.95 (s, 1H), 7.12 (s, 1H), 7.16-7.21 (m, 3H), 7.46-7.49 (m, 1H), 7.63 (s, 1H), 7.66 (dd, *J* = 5.3, 8.6 Hz, 2H), 7.74 (dd, *J* = 1.8, 8.7 Hz, 1H), 7.91 (d, *J* = 8.6 Hz, 1H), 7.93 (d, *J* = 9.0 Hz, 1H), 8.04 (d, *J* = 1.6 Hz, 1H); δ_C ($CDCl_3$, 125 MHz) 48.8, 115.4, 115.8, 115.9, 119.4, 119.5, 123.1, 126.0, 126.3, 126.4, 126.5, 126.6, 128.9, 129.0, 129.5, 129.9, 131.1, 134.1, 136.5, 138.0, 141.2, 141.9, 162.7 (d, *J* = 247.1 Hz, C-F); MS (ESI): *m/z* = 303.1 [M^++H].

1-((6-(4-Fluorophenyl)-2-methoxynaphthalen-1-yl)methyl)-1*H*-imidazole, 7. Synthesised according to Method D using **7a** (0.10 g, 0.35 mmol) and CDI (0.12 g, 0.71 mmol) in acetonitrile as solvent; yield: 0.06 g (49 %); light-yellow solid: mp 128 °C; R_f = 0.52 (DCM / MeOH, 95:5); IR (ATR) υ (cm^{-1}) 1601 (w), 1503 (s), 1257 (s), 1235 (s), 1162 (w), 1096 (s), 1075 (w), 1056 (w), 1026 (w), 904 (w), 844 (w), 823 (w), 788 (w), 733 (w), 662 (m), 623 (m), 551 (m), 505 (s); δ_H ($CDCl_3$, 500 MHz) 4.00 (s, 3H), 5.58 (s, 2H), 6.97 (d, *J* = 10.7 Hz, 2H), 7.14-7.18 (m, 2H), 7.34 (d, *J* = 9.1 Hz, 1H), 7.58 (s, 1H), 7.63 (dd, *J* = 5.3, 8.7 Hz, 2H), 7.73 (dd, *J* = 1.9, 8.8 Hz, 1H), 7.93 (d, *J* = 9.1

Hz, 1H), 7.96 (d, *J* = 1.8 Hz, 1H), 7.99 (d, *J* = 8.9 Hz, 1H); δ_C ($CDCl_3$, 125 MHz) 40.6, 56.3, 113.3, 115.6, 115.7, 115.8, 119.0, 122.5, 126.4, 127.1, 128.6, 128.7, 128.9, 129.2, 131.1, 131.9, 135.5, 136.6, 137.1, 155.7, 162.5 (d, *J* = 246.7 Hz, C-F); MS (ESI): *m/z* = 333.1 [M^++H].

1-((6-(4-Fluorophenyl)naphthalen-2-yl)methyl)-1*H*-imidazole, 8. Synthesised according to Method D using **8a** (0.15 g, 0.60 mmol) and CDI (0.50 g, 3.08 mmol) in acetonitrile as solvent; yield: 0.12 g (67 %); white solid: mp 114 °C; R_f = 0.50 (DCM / MeOH, 95:5); IR (ATR) υ (cm^{-1}) 3099 (w), 1603 (m), 1503 (s), 1439 (w), 1293 (w), 1231 (s), 1160 (w), 1105 (w), 1089 (w), 910 (m), 842 (m), 816 (s), 772 (w), 657 (s), 558 (m), 519 (w); δ_H ($CDCl_3$, 500 MHz) 5.29 (s, 2H), 6.96 (s, 1H), 7.13 (s, 1H), 7.15-7.19 (m, 2H), 7.29 (dd, *J* = 1.5, 8.5 Hz, 1H), 7.61 (s, 1H), 7.64-7.67 (m, 3H), 7.72 (dd, *J* = 1.8, 8.5 Hz, 1H), 7.85-7.88 (m, 2H), 7.97 (s, 1H); δ_C ($CDCl_3$, 125 MHz) 51.0, 115.7, 115.9, 119.4, 125.4, 126.1, 126.2, 128.5, 128.9, 129.0, 129.2, 129.8, 132.3, 133.2, 133.7, 136.85, 136.88, 137.5, 138.3, 162.6 (d, *J* = 246.9 Hz, C-F); MS (ESI): *m/z* = 302.9 [M^++H].

1-((3-(4-Fluorophenyl)naphthalen-1-yl)methyl)-1*H*-imidazole, 9. Synthesised according to Method D using **9a** (0.15 g, 0.60 mmol), CDI (0.39 g, 2.38 mmol) and imidazole (0.04 g, 0.60 mmol); yield: 0.13 g (71 %); light-yellow oil; R_f = 0.23 (DCM / MeOH, 98:2); IR (ATR) υ (cm^{-1}) 3057 (w), 1606 (w), 1511 (s), 1229 (s), 1160 (m), 1107 (w), 1077 (w), 832 (s), 790 (w), 749 (m), 663 (m), 519 (w); δ_H ($CDCl_3$, 500 MHz) 5.64 (s, 2H), 6.97 (s, 1H), 7.13 (s, 1H), 7.14-7.17 (m, 2H), 7.38 (s, 1H), 7.53-7.58 (m, 2H), 7.60 (dd, *J* = 5.3, 8.6 Hz, 2H), 7.69 (s, 1H), 7.87-7.89 (m, 1H), 7.94-7.96 (m, 1H), 8.00 (s, 1H); δ_C ($CDCl_3$, 125 MHz) 48.9, 115.7, 115.8, 116.0, 119.5, 122.2, 123.6, 124.7, 125.7, 126.3, 126.79, 126.82, 127.1, 127.2, 128.8, 128.9, 129.3, 129.5, 129.9, 131.9, 132.7, 134.1, 136.27, 136.29, 137.2, 162.7 (d, *J* = 247.4 Hz, C-F); MS (ESI): *m/z* = 303.0[M^++H].

1-(3-(6-Methoxynaphthalen-2-yl)benzyl)-1*H*-imidazole, 16. Synthesised according to Method D using **7a** (0.20 g, 0.76 mmol) and CDI (0.38 g, 2.27 mmol) in acetonitrile as solvent; yield: 0.12 g (51 %); white solid: mp 130 °C; R_f = 0.38 (DCM / MeOH, 95:5); IR (ATR) υ (cm^{-1}) 2966 (w), 1626 (w), 1603 (w), 1492 (w), 1453 (w), 1388 (w), 1256 (m), 1227 (m), 1212 (m), 1073 (m), 1022 (s), 883 (w), 855 (s), 814 (s), 791 (m), 745 (s), 662 (s); δ_H ($CDCl_3$, 500 MHz) 3.94 (s, 3H), 5.20 (s, 2H), 6.97 (s, 1H), 7.13-7.16 (m, 3H), 7.19 (dd, *J* = 2.5, 8.9 Hz, 1H), 7.44-7.47 (m, 1H), 7.48-7.49 (m, 1H), 7.63-7.66 (m, 3H), 7.78-7.81 (m, 2H), 7.92 (d, *J* = 1.5 Hz, 1H); δ_C ($CDCl_3$, 125 MHz) 51.0, 55.3, 105.7, 119.3, 125.7, 125.8, 125.9, 126.1, 127.2, 127.4, 129.1, 129.5, 129.7, 134.0, 135.5, 136.7, 142.1, 158.0; MS (ESI): *m/z* = 315.0 [M^++H].

1-(4-(6-(*tert*-Butyldimethylsilyloxy)naphthalen-2-yl)benzyl)-1*H*-imidazole, 17a. Synthesi-sed according to Method D using **17b** (3.46 g, 9.50 mmol) and CDI (7.70 g, 47.40 mmol). Then the desired product was taken to the next step without further purification; yield: 3.11 g (79 %); white solid; R_f = (DCM / MeOH, 98:2).

6-Bromo-2-methoxy-1-naphthaldehyde, 7c. To a solution of 2-bromo-6-methoxy-naphthalene (5.00 g, 21.09 mmol) in DCM (100 mL) was added $TiCl_4$ (4.00 g, 21.09 mmol) and 1,1-dichloromethylmethylether (2.43 g, 21.09mmol). Then the resulting mixture was stirred at 40 °C for 2 hours. After complete conversion, water (25 mL) was added, the phases were separated, and the aqueous layer 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 taken to the next step without further purification; yield: g (100 %); white solid.

Method E: Reduction with $NaBH_4$. To an ice-cooled solution of the corresponding aldehyde (1 eq) in THF (5 mL / mmol) was added $NaBH_4$ (2 eq) in methanol (5 mL / mmol). Then the resulting mixture was stirred at rt for 1 hour. After complete conversion, the solvent was distilled off under reduced pressure. Then water (25 mL) was added, and 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.

(6-(4-Fluorophenyl)-2-methoxynaphthalen-1-yl)methanol, 7a. Synthesised according to Method E using **7b** (0.28 g, 0.98 mmol) and $NaBH_4$ (0.08 g, 1.96 mmol); yield: 0.25 g (91 %); white solid; R_f = 0.56 (DCM / MeOH, 98:2); δ_H ($CDCl_3$, 500 MHz) 4.01 (s, 3H), 5.21 (s, 2H), 7.15-7.18 (m, 2H), 7.33 (d, *J* = 9.1 Hz, 1H), 7.66 (dd, *J* = 5.3, 8.9 Hz, 2H), 7.74 (dd, *J* = 1.9, 8.9 Hz, 1H), 7.89 (d, *J* = 9.1 Hz, 1H), 7.95 (d, *J* = 1.9 Hz, 1H), 8.18 (d, *J* = 8.9 Hz, 1H); δ_C ($CDCl_3$, 125 MHz) 55.9, 56.5, 113.5, 115.6, 115.8, 121.2, 123.7, 126.1, 126.5, 128.66, 128.72, 129.4, 130.3, 131.9, 135.3, 136.98, 137.00, 155.3, 162.4 (d, *J* = 246.5 Hz, C-F); MS (APCI): m/z = 266.9 [M^++H].

(3-(6-Methoxynaphthalen-2-yl)phenyl)methanol, 16a. Synthesised according to Method E using **16b** (0.40 g, 1.53 mmol) and $NaBH_4$ (0.12 g, 3.05 mmol); yield: 0.40 g (100 %); white solid; R_f = 0.50 (DCM / MeOH, 98:2); MS (EI): m/z = 312.9, 316.0 [M^++H].

(4-(6-(*tert*-Butyldimethylsilyloxy)naphthalen-2-yl)phenyl)methanol, 17b. Synthesised according to Method E using **17c**. (4.55 g, 12.50 mmol) and $NaBH_4$ (0.95 g, 25.00 mmol). Then the desired product was taken to the next step without further purification; yield: 3.46 g (76 %); white solid; R_f = 0.35 (hexane / EtOAc, 70:30).

(6-Bromonaphthalen-2-yloxy)(*tert*-butyl)dimethylsilane, 17d. To a solution of 6-bromonaphthalen-2-ol (10.00 g, 44.80 mmol) and imidazole (3.40 g, 49.30 mmol) in DCM (100 mL) was added dropwise a TBDMS-Cl (7.40 g, 49.30 mmol) solution in DCM (50 mL). Then the resulting mixture was stirred at rt for 16 hours. After complete conversion, water (25 mL) was added, the phases separated, and the aqueous layer 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 taken to the next step without further purification; yield: 14.81 g (98 %); white solid.

6-(4-((1*H*-Imidazol-1-yl)methyl)phenyl)naphthalen-2-ol, 17. To a solution of **17a** (3.11 g, 7.50 mmol) in THF (50 mL) was added dropwise a 1 M TBAF solution in THF (7.50 mL, 7.50 mmol). Then the resulting mixture was stirred at rt for 16 hours. After complete conversion, water (25 mL) and ethyl acetate (25 mL) were added, the phases separated, and the aqueous layer 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; yield: 1.96 g (87 %); brown solid; R_f = 0.50 (EtOAc / MeOH, 95:5); IR (ATR) υ (cm^{-1}) 3113 (w), 3026 (w), 1603 (m), 1516 (m), 1235 (s), 1198 (s), 1108 (m), 1080 (m), 831 (s), 806 (s); δ_H ($CDCl_3$, 500 MHz) 5.21 (s, 2H), 6.90 (t, *J* = 1.0 Hz, 1H), 7.08 (dd, *J* = 2.4 Hz, *J* = 8.7 Hz, 1H), 7.11 (d, *J* = 2.3 Hz, 1H), 7.20 (t, *J* = 1.2 Hz, 1H), 7.33 (d, *J* = 8.4 Hz, 2H), 7.67 (dd, *J* = 1.8 Hz, *J* = 8.6 Hz, 1H), 7.70-7.74 (m, 3H), 7.77 (t, *J* = 0.9 Hz, 1H), 7.79 (d, *J* = 8.9 Hz, 1H), 8.03 (d, *J* = 1.3 Hz, 1H), 9.82 (s, 1H); δ_C ($CDCl_3$, 125 MHz) 49.1 (CH_2), 108.4, 119.0, 119.5, 125.0, 125.1, 126.7, 126.8, 127.9, 128.1, 128.7 (C_q), 129.8, 133.7 (C_q), 133.8 (C_q), 136.5 (C_q), 137.4 , 139.6 (C_q), 155.5 (C-OH); MS (ESI): m/z = 301.0 [M^++H].

Molecular Modelling. Compound **15** was 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 pyridine 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. 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 compound **15** were clustered with ACIAP[36, 37] 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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V. Synthesis, Biological Evaluation, and Molecular Modeling of Abiraterone Analogues: Novel CYP17 Inhibitors for the Treatment of Prostate Cancer

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J. Med. Chem. **2008**, *51*, 5009–5018

Abstract

Abiraterone, a steroidal cytochrome P450 17α-hydroxylase-17,20-lyase inhibitor (CYP17), is currently undergoing phase II clinical trials as a potential drug for the treatment of androgen-dependent prostate cancer. Since steroidal compounds often show side effects attributable to their structure, we have tried to replace the sterane scaffold by nonsteroidal core structures. The design and synthesis of 20 new abiraterone mimetics are described. Their activities have been tested with recombinant human CYP17 expressed in *E. coli*. Promising compounds were further evaluated for selectivity against CYP11B1, CYP11B2, and the hepatic CYP3A4. Compounds **19** and **20** showed comparable activity to abiraterone (IC_{50} values of 144 and 64 nM vs 72 nM) and similar or even better selectivity against the other CYP enzymes. Selected compounds were also docked into our homology model, and the same binding modes as for abiraterone were found.

Introduction

Prostate cancer (PC) is the most common tumor and age-related cause of death in elder men worldwide.[1] Because of the advanced age of the patients, less invasive approaches are needed. Accordingly, the treatment of choice is 'watchful waiting,'[2] followed by radiation therapy only when it is necessary. Since PC is androgen dependent in over 80% of the cases, another current standard treatment is orchiectomy, the surgical removal of the testes, usually applied to patients under 70 years old. The reduction of testicular androgen production by gonadotropin-releasing hormone (GnRH) analogues,[3] a ‘medical castration’, is often preferred over the surgical one and can also be used for treating older patients. Nevertheless, castration whether surgical or medical reduces maximally 90-95% of the daily testosterone production, which is often not enough to stop the tumor from growing, since prostate levels of testosterone and dihydrotestosterone are still about 25% and 10%, respectively, even after three months of treatment with a GnRH agonist.[4]

The remaining 5-10% of the androgens are produced in the adrenals. In the 1980s, Labrie[5] hypothesized that additionally counteracting adrenal androgens by application of antiandrogens would further inhibit tumor growth. This approach, known as ‘combined androgen blockade’ (CAB), has been widely used in the past. The results have been partially positive, especially in patients with minimal disease and good performance status. However, it must be mentioned that antiandrogen therapy is associated with notable side effects.[6] Another drawback of CAB is that in refractory PC regression is observed after discontinuation of antiandrogen administration. This led to the hypothesis that under antiandrogen treatment androgen receptor mutations occur causing PC cells to recognize antagonists as agonists.[7]

An alternative target proposed in the recent past is the cytochrome P450 enzyme 17α-hydroxylase-17,20-lyase (CYP17). This enzyme is localized in the endoplasmic reticulum in the testes as well as in the adrenals and is the key enzyme for androgen biosynthesis. Its inhibition should stop the production of androgens both in the testes and in the adrenals, and therefore, inhibitors of CYP17 should be more effective for treating androgen-dependent PC than GnRH analogues. Proof of principle was achieved by the unspecific CYP inhibitor ketoconazole, which clinically turned out to be a good adjuvant therapeutic capable of reducing testosterone levels.[8] Nevertheless, the side effects shown by this antimycotic compound are the reason that it is not used anymore.[9]

CYP17 catalyzes two reactions, the 17α-hydroxylation of pregnenolone and progesterone to the corresponding 17α alcohols and the subsequent 17,20-lyase reaction cleaving the C17-C20 bond. This yields the 17-keto androgens androstendione and dehydroepiandrosterone, precursors of all other androgens, including testosterone (Chart 1).

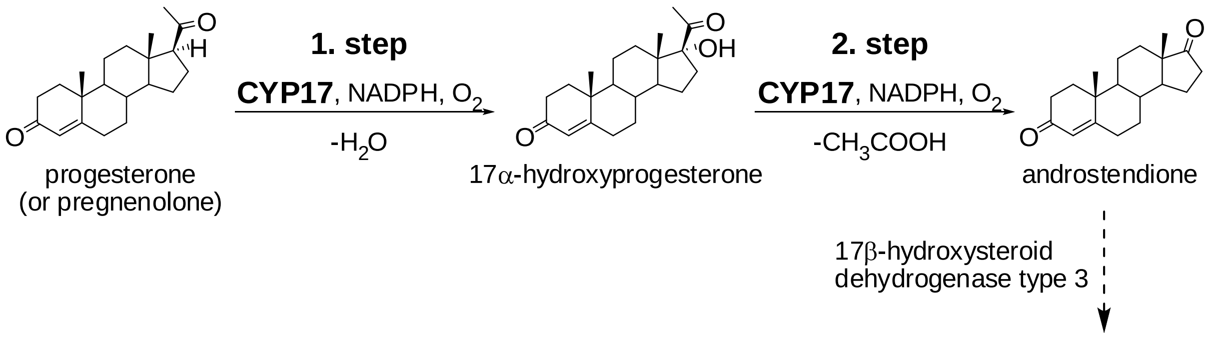


Chart 1. The role of CYP17 in androgen biosynthesis

The side effects of ketoconazole caused others[10] and our group to develop steroidal[11] and nonsteroidal[12,13] inhibitors. One compound, the steroidal CYP17 inhibitor abiraterone (Chart 2), is currently undergoing phase II clinical trials showing high activity in postdocetaxel castration refractory PC patients. In contrast to ketoconazole, it seems to have no dose-limiting toxicity.[14]

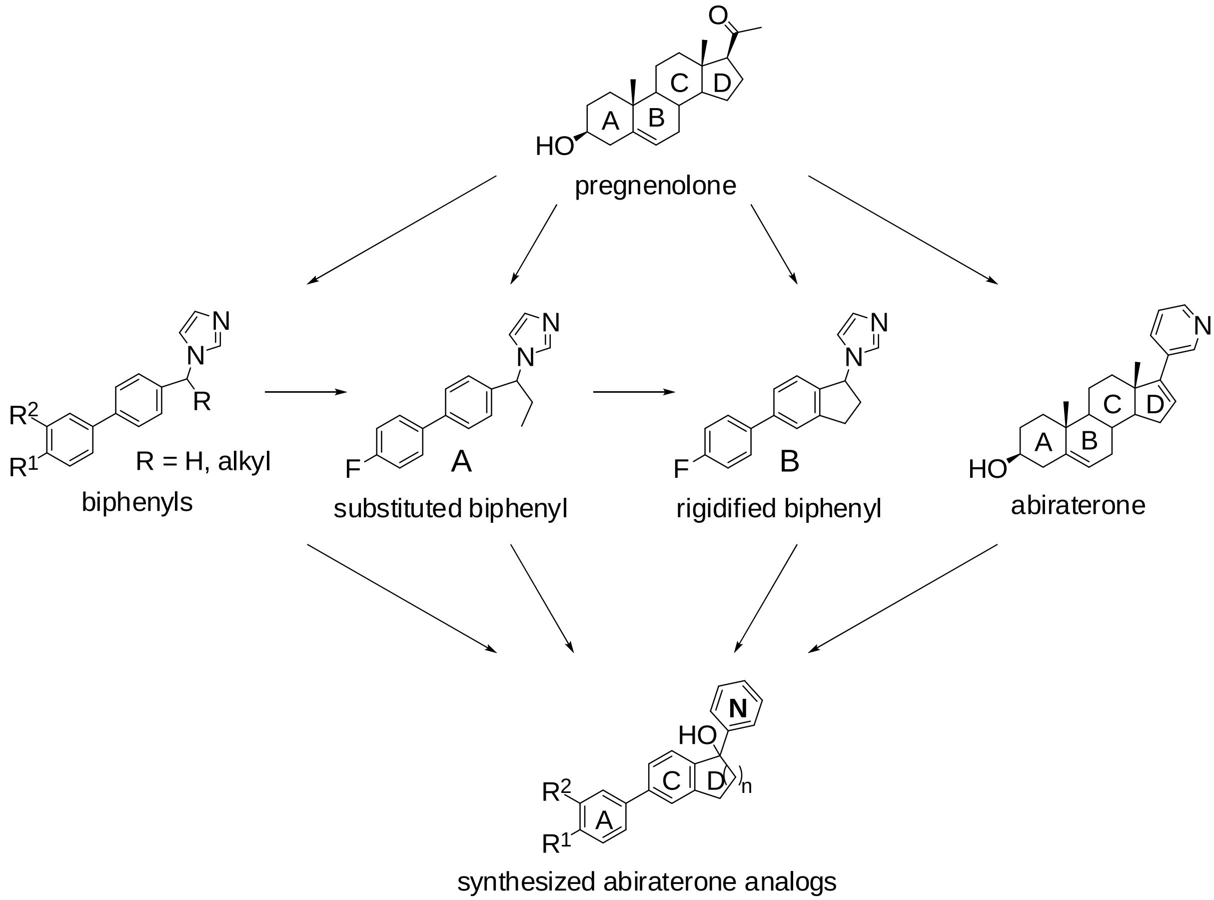
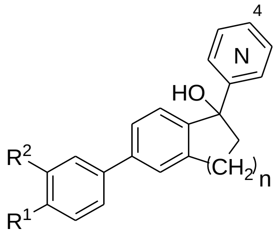


Chart 2. Substrate, abiraterone and synthesized mimetics

Because steroidal compounds often show side effects due to interactions with steroid receptors,[15] it is our aim to develop nonsteroidal compounds. In this work, we present the design, synthesis, *in vitro* evaluation regarding activity and selectivity and modeling studies of nonsteroidal analogues of abiraterone (Chart 3).



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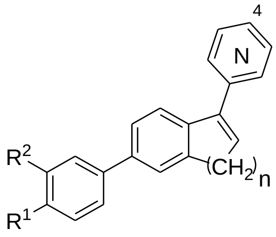

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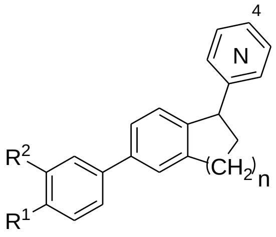


15-20

Compound	R^1	R^2	n	N
1	F	H	1	3
2	F	H	1	4
3	OMe	H	1	4
4	OMe	F	1	4
5	F	H	2	4
6	F	F	2	4
7	OMe	F	2	4
8	F	H	1	3
9	F	H	1	4
10	OMe	H	1	4
11	F	H	2	4
12	F	F	2	4
13	OH	OH	2	4
14	OH	F	2	4
15	F	H	1	3
16	F	H	1	4
17	F	H	2	4
18	F	F	2	4
19	OH	OH	2	4
20	OH	F	2	4

Chart 3. List of synthesized compounds **1**-**20**

Design

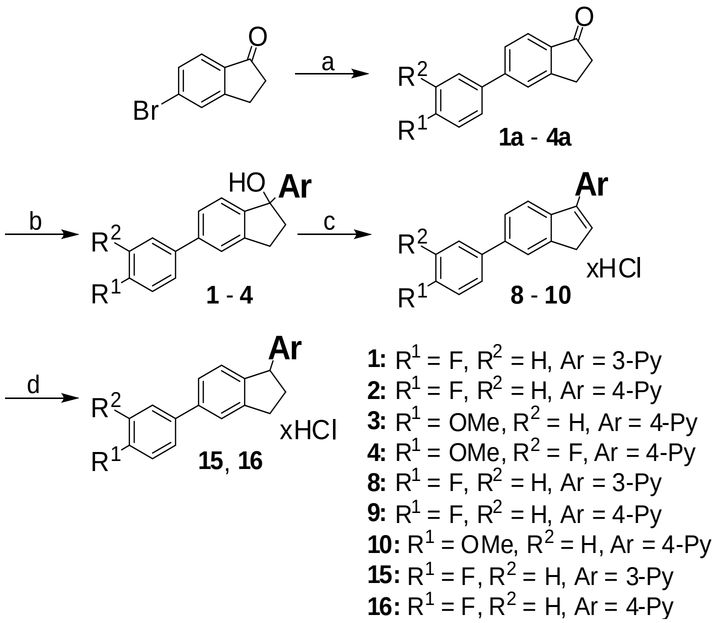
The three most important structural features of abiraterone are the aromatic nitrogen-containing heterocycle, the hydrophobic steroidal core, and the hydroxy group mimicking the oxygen at the 3-position of the substrates. All of them are similarly important for a high affinity to the enzyme. CYP enzymes have the particularity of bearing a heme moiety in their binding site. The complexation of the Fe^{2+} of the heme with an sp^2 hybridized nitrogen as in pyridyl and imidazolyl is widely described.[16] In recent studies[13] we showed that a biphenyl moiety can replace the steroidal core AC-rings and that a biphenyl moiety furnished with an imidazolyl-methylene group ensures high CYP17 inhibitory activity (Chart 2). Introduction of alkyl substituents at the methylene bridge[13b,f] (for example, compound **A**[13f]) and first attempts to rigidify the structures resulting in indane compounds (like compound **B**[13b]) increased activity. Interestingly, 4-fluoro substituted compounds in general showed very high inhibitory activity. Compound **A**, the indane ring opened analogue to compound **B**, was more active in the rat and showed a longer plasma half-life and higher bioavailability compared to abiraterone, while its *in vitro* activity with regard to potency and selectivity was not as outstanding as that of the steroidal compound. In this work we want to elucidate whether it is possible to further increase activity and especially selectivity by additional attempts to rigidify the biphenyl structure and to exchange the heterocycle. The resulting compounds are mimics of the steroidal compound abiraterone. For the design of the synthesized compounds, the steroidal core was replaced by an indane (mimicking the steroidal C- and D-rings) or tetrahydronaphthalene scaffold (for expansion of the D-ring) connected to an A-ring mimicking phenyl moiety. Electron donating and withdrawing substituents (including hydroxy and fluoro) were introduced in different positions at the phenyl ring. The iron-complexing aromatic nitrogen was represented by a 3- or 4-pyridine moiety.

Three classes of mimetically relevant compounds were synthesized. Compounds **1**-**7**, hydroxylated at the C17 mimicking position, are analogues of the steroidal substrates for the second enzymatic step (lyase reaction). Compounds **7**-**14** bearing a double bond are structurally analogous to abiraterone. The saturated alkanes **15**-**20** show the highest similarity to the first-step (hydroxylase reaction) substrates.

In the following, the synthesis, determination of activity, and selectivity and molecular modeling studies of the synthesized abiraterone analogues are presented. Besides CYP17 inhibitory activity, inhibition of other CYP enzymes was examined to exclude possible side effects due to unspecific heme iron complexation. Thus, selectivity toward CYP11B1 and CYP11B2 was determined, since their biological relevance relies on the fact that CYP11B1 is involved in glucocorticoid biosynthesis while CYP11B2 catalyzes the last step in mineralocorticoid formation. Special attention was given to the most crucial hepatic enzyme CYP3A4, since it is involved in the metabolism of over 50% of all prescription drugs.[17] The most interesting compounds were docked into our homology-approach derived protein model and the binding modes in the active site discussed.

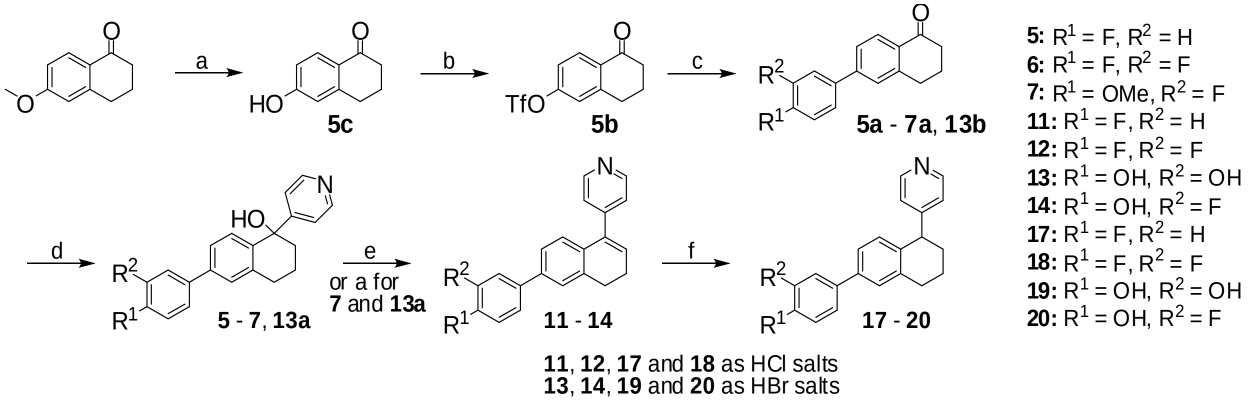
Chemistry

The syntheses of compounds **1**-**20** are shown in Schemes 1 and 2. In our aim to rigidify our biphenylic core structure by connecting the methylene bridge with the C-ring mimicking phenyl moiety, we synthesized indane derivatives (Scheme 1), which can be considered abiraterone analogues, and expanded the nonaromatic ring by one methylene group yielding tetrahydronaphthalene derivatives (Scheme 2). Different substitution patterns at the A-ring mimicking phenyl substituent were used. Suzuki coupling[18] (Method A) using 5-bromoindanone for the abiraterone analogues and 1-tetralon-6-yl trifluoromethanesulfonate, synthesized from the respective alcohol, for the tetrahydronaphthalenes yielded the indanones **1a**-**4a** and the tetralones **5a**-**7a**. The pyridine moiety was introduced by means of a nucleophilic addition (Method B) with the corresponding lithium pyridinyl salt to yield the tertiary alcohols **1**-**7** and **13a**. The condensation to the corresponding alkenes was performed in HCl/isopropanol (**8**-**12**, Method C) or in HBr/H2O (**13** and **14**, Method E). The latter method was applied when a simultaneous ether cleavage was desired. The hydration to the saturated rings was carried out with Pearlman's catalyst $(Pd(OH)_2)$, resulting in compounds **15**-**20** (Method D).



Scheme 1 (left). Synthesis of indane derivatives
Reagents and conditions: a: Method A: Na_2CO_3, $R^1R^2C_6H_3B(OH)_2$, $Pd(PPh_3)_4$, toluene, H_2O, reflux, 8 h; **b: Method B:** 3-iodopyridine or 4-bromopyridine, *n*-BuLi, THF, Et_2O, -78 °C - rt, 3h; **c: Method C:** HCl in *i*-propanol, rt, 2h; **d: Method D:** $Pd(OH)_2$, ethanol, THF, H_2, rt, 3h.

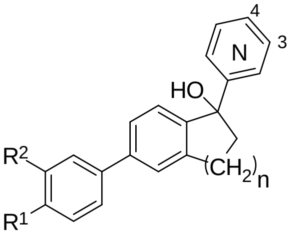
Scheme 2 (down). Synthesis of tetrahydronaphthalene derivatives
Reagents and conditions: a: Method E: HBr, reflux, 16 h; **b:** pyridine, Tf_2O, DCM, 0 °C - rt, 3h; **c: Method A:** Na_2CO_3, $R^1R^2C_6H_3B(OH)_2$, $Pd(PPh_3)_4$, toluene, H_2O, reflux, 8 h; **d: Method B:** 3-iodopyridine or 4-bromopyridine, *n*-BuLi, THF, Et_2O, -78 °C - rt, 3h; **e: Method C:** HCl in *i*-propanol, rt, 2h; **f: Method D:** $Pd(OH)_2$, ethanol, THF, H_2, rt, 3h.



Results

Biological Results. Inhibition of human CYP17 was determined by performing our previously described assay.[13d] For the source of human CYP17, *E. coli*[19] coexpressing human CYP17 and NADPH-P450 reductase were used. After homogenization, the 50 000 g sediment was incubated with progesterone (25 µM) and the inhibitor.[12a] Separation of the product was performed by HPLC using UV detection. The IC$_{50}$ values determined for compounds **1**-**20** are shown in Table 1.

Table 1. Inhibition of CYP17 by alcohols **1**-**7**, alkenes **8**-**4** and alkanes **15**-**20**



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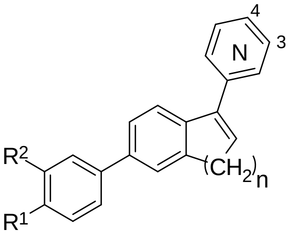

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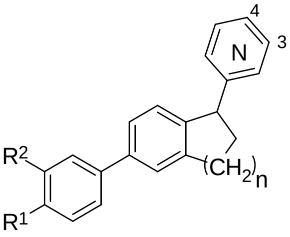


15-20

Structures [a]				**CYP17 IC$_{50}$ [nM]** [b]					
R^1	**R^2**	**n**	**N**	**Comp.**	**Alcohols**	**Comp.**	**Alkenes**	**Comp.**	**Alkanes**
Indanes									
F	H	1	3	**1**	> 20,000	**8**	2346	**15**	> 20,000
F	H	1	4	**2**	333	**9**	> 20,000	**16**	233
OMe	H	1	4	**3**	> 20,000	**10**	> 5,000		
OMe	F	1	4	**4**	> 10,000				
Hydronaphthalenes									
F	H	2	4	**5**	587	**11**	> 5,000	**17**	163
F	F	2	4	**6**	423	**12**	> 5,000	**18**	1222
OMe	F	2	4	**7**	> 5,000				
OH	OH	2	4			**13**	307	**19**	144
OH	F	2	4			**14**	188	**20**	64
	KTZ [c]						2780		
	ABT [d]						72		

[a] Compounds **8**-**12** and **15**-**18** were tested as HCl salts and **13**, **14**, **19** and **20** as HBr salts

[b] Data shown were obtained by performing the tests in duplicate. The deviations were within < ±5 %. Concentration of progesterone (substrate) was 25 µM

[c] **KTZ**: ketoconazole

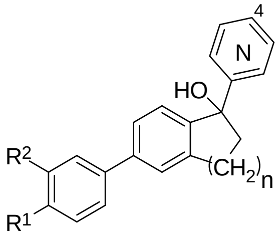
[d] **ABT**: abiraterone

The compounds can be divided into two classes according to the CD-ring mimicking moiety, namely in indane and hydronaphthalene derivatives, and also into three classes regarding the pyridyl bearing C atom, that is in alcohols, alkenes, and alkanes. Most of the indane derivatives, either bearing a 3-pyridyl or a 4-pyridyl substituent, showed no or little CYP17 inhibition. Only compound **2**, with a moderate inhibition of 333 nM, and compound **16** (IC_{50} = 233 nM) were active. Interestingly, both compounds contain a 4-pyridyl group as iron-complexing heterocycle and a fluorine at R^1.

The hydronaphthalenes were more potent than the indanes, showing a broad range of activity. The alkenes (**11**-**14**) were weaker inhibitors than the corresponding compounds with a single bond, the alcohols (**5**-**7**) and tetrahydronaphthalenes (**17**-**20**). The inhibition values range from about 200 to over 5000 nM, while for the single bond compounds the values range mostly from around 100 to 500 nM. An important condition for inhibitory activity in the class of the alkanes is a 4-pyridyl substituent. In the 4-position of the phenyl ring (R^1) a F or OH group and in 3-position (R^2) H, F, or OH led to highly active compounds. Interestingly, the 3,4-di-F-substituted compound **18** only showed moderate activity. One explanation for this finding might be the electron withdrawing effect of the two F atoms at the phenyl ring. The most potent compounds were the 4-OH compounds **19** and **20** (IC_{50} = 144 and 64 nM). A hydroxy substituent in 4-position of the phenyl ring also makes the alkenes from the 4-pyridyl substituted dihydronaphthalene type potent inhibitors (compounds **13** and **14**). In the class of the indenes, 3-pyridyl substitution, as in abiraterone, led to an active compound (**8**) whereas 4-pyridyl substitution does not (**9**). It is striking, that 11 out of the 20 synthesized compounds were more active than ketoconazole. The most potent compound **20** showed a slightly higher inhibition compared to abiraterone (IC_{50} = 72 nM). Our seven most potent compounds from this work were more active than compound **A** (345 nM), and regarding compound **B** (670 nM), nine showed significantly higher inhibitory activities for CYP17. In the whole cell assay, i.e., *E. coli* coexpressing human CYP17 and NADPH-P450 reductase,[12f] the most active compound of this series, **20**, was also highly active (IC_{50} < 200 nM).

Since the target enzyme contains a heme in its binding site, hence CYP/P450 enzyme, which is crucial for the enzymatic reaction and for binding the iron-complexing nitrogen containing inhibitors, the latter compounds were tested for selectivity against other CYP enzymes. Accordingly, selected compounds were tested for inhibitory activity on the steroidogenic CYP enzymes CYP11B1 and CYP11B2, key enzymes in glucocorticoid and mineralocorticoid biosynthesis. For the assay,[20] V79MZh11B1 cells expressing human CYP11B1 and V79MZh11B2 cells expressing human CYP11B2 were used. The IC_{50} values determined for selected compounds are shown in Table 2.

Table 2. Inhibition of CYP11B1, CYP11B2 and CYP3A4 by compounds **1**-**20**



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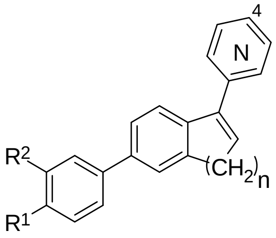

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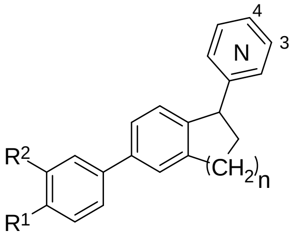

15-20

Comp. [a]	**Structures**				**CYP11B1** [b]	**CYP11B2** [b]	**CYP3A4** [b]
	R^1	**R^2**	**n**	**N**	**IC_{50} [nM]**	**IC_{50} [nM]**	**IC_{50} [nM]**
1	F	H	1	3	n.d. [c]	n.d.	> 20,000
2	F	H	1	4	> 10,000	816	3231
3	OMe	H	1	4	n.d.	n.d.	3291
4	OMe	F	1	4	291	436	3364
5	F	H	2	4	1159	840	8757
6	F	F	2	4	3109	1676	13841
7	OMe	F	2	4	686	945	15190
8	F	H	1	3	n.d.	n.d.	6947
9	F	H	1	4	n.d.	n.d.	159
10	OMe	H	1	4	n.d.	n.d.	2394
11	F	H	2	4	> 10,000	121	4199
12	F	F	2	4	> 20,000	311	3643
13	OH	OH	2	4	> 10,000	1492	357
14	OH	F	2	4	2748	991	2114
15	F	H	1	3	n.d.	n.d.	1697
16	F	H	1	4	1076	543	1093
17	F	H	2	4	> 5,000	518	2173
18	F	F	2	4	> 20,000	567	2300
19	OH	OH	2	4	2135	2324	632
20	OH	F	2	4	1370	587	3386
		KTZ [d]			127	67	72
		ABT [e]			1608	1751	2704

[a] Compounds **8**-**12** and **15**-**18** were tested as HCl salts and **13**, **14**, **19** and **20** as HBr salts

[b] Data shown were obtained by performing the tests in duplicate. The deviations were within < ±5 %. Concentration of progesterone (substrate) was 25 µM

[c] n.d.: not determined

[d] **KTZ**: ketoconazole

[e] **ABT**: abiraterone

With the exception of compounds **4** and **7** showing IC_{50} values of 291 and 686 nM toward CYP11B1, respectively, the other tested compounds exhibited IC_{50} values above 1 µM. Most of the compounds (**2**, **6**, **11**-**14**, **17**-**19**) turned out to be more selective than abiraterone (IC_{50} = 1608 nM), and all compounds were more selective compared to ketoconazole (127 nM) regarding the cortisol forming enzyme. Concerning compound **A** (66% inhibition at 0.2 µM), all compounds from this study showed much higher selectivity.

The inhibition of CYP11B2 observed with the evaluated compounds was a little higher showing IC_{50} values between 121 and 2324 nM. Compared to abiraterone (IC_{50} = 1751 nM), compounds **6** and **13** exhibited a similar selectivity whereas compound **19** (IC_{50} = 2324 nM) was more selective than the reference. Compared to ketoconazole (IC_{50} = 67 nM) and compound **A** (66% inhibition at 0.2 µM), all compounds were more selective with the exception of compound **11** (IC_{50} = 121 nM).

The synthesized compounds were also evaluated for inhibition of the most crucial hepatic CYP enzyme CYP3A4 in regard to its important role in drug metabolism. Except for compound **9** (IC_{50} = 159 nM) and compound **13** (IC_{50} = 357 nM), the IC_{50} values for compounds **1**-**20** were between 632 and >20000 nM. These compounds were more selective than ketoconazole (72 nM) and compound **A** (88% at 1 µM). Most of the compounds were even more selective than abiraterone (IC_{50} = 2704 nM).

Molecular Modeling. Since there is no crystal structure of CYP17 available, we recently built a homology model using the X-ray structure of human CYP2C9 (PDB ID 1r9o) as template.[13e] Docking simulations with energy minimized compounds were carried out by means of the GOLD 3.2 software running Linux CentOS 5.1 on an Intel® P4 CPU 3.00 GHz computer, using a slightly modified GOLDSCORE function with goldscore.P450_pdb parameters, for a better evaluation of hydrophobic interactions. From every class, the most potent compounds were docked into this protein model, and in the case of chiral compounds, both enantiomers were docked.

In Figure 1 two binding modes of the substrate pregnenolone are shown: one already described by others[21] (SM1 mode) and an additional one (SM2). Poses in SM1 occur more often (approximately 7:3) with an overall better scoring with respect to those in SM2. Furthermore, pregnenolone was also docked with CHEMSCORE, a scoring function with a higher assessment of the hydrophobic interactions, showing poses mostly in SM1 (>9:1). Interestingly, great similarities between SM2 and the orientation of our previously described biphenyl compound **A** can be observed (there are actually two very similar modes described as BM1 and BM2[13e]). Like the substrate, the steroidal inhibitor abiraterone is also able to bind in both modes but showing a good complexation with the heme iron only in SM1 (Figure 1). The same is true for compound **B**, the ring-closed analogue of compound **A** (not shown).

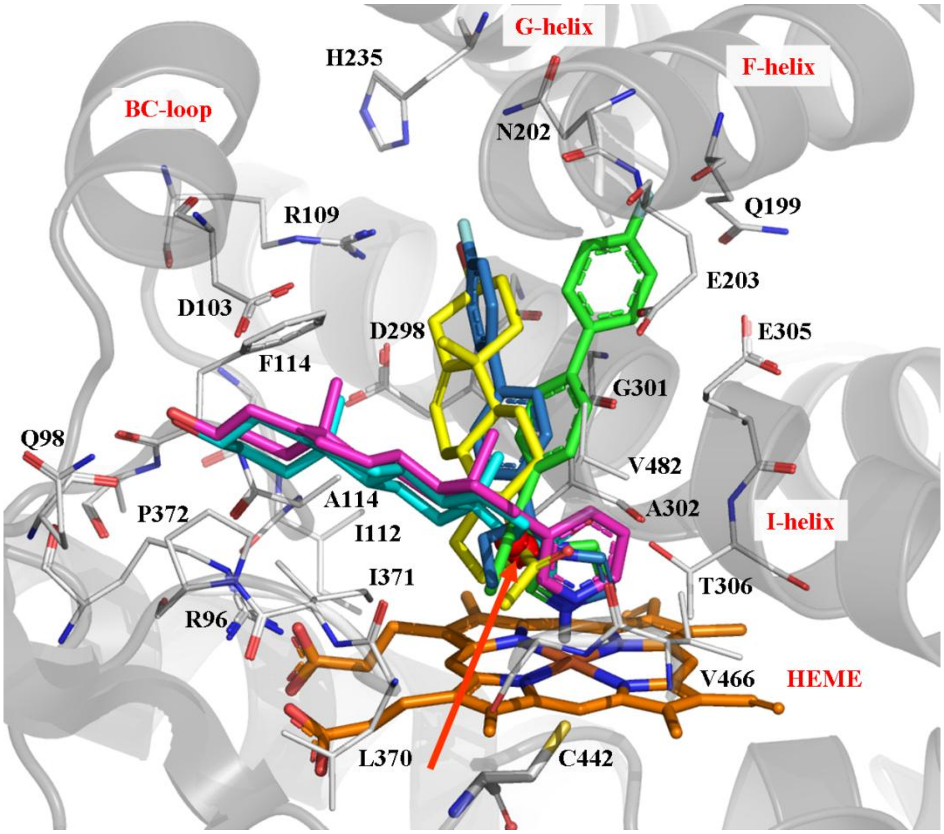

Figure 1. Both binding modes of the substrate pregnenolone (SM1, cyan, and SM2, yellow; the red arrow indicates the position of the C17 atoms, rendered as red spheres) are shown as docking complex with CYP17. For comparison, the two orientations of compound A (in the formerly described binding modes BM1, blue, and BM2, green[13f]) and abiraterone (magenta) are given. Heme, interacting residues and ribbon rendered tertiary structure of the active site are also represented. Figures were generated with Pymol (http://www.pymol.org).

The *R* and *S* configurated abiraterone analogues of the alcohol and alkane class also bind in both modes. Interestingly, a correlation between the inhibitory activities of the compounds of each class and the score values could be observed. In the case of highly active compounds, the percentage of poses presenting a good iron-nitrogen coordination is higher than for weak inhibitors. For enantiomers of a given racemic compound, one enantiomer always shows a preference for poses in one binding mode while the other enantiomer prefers to dock in the alternative one. In the case of the alcohols, in Figure 2 exemplified by compound **5**, the *R* enantiomer binds predominantly in the SM1 mode (9:1) while the *S* enantiomer binds in SM2 (8:2). The opposite relation can be seen for the alkanes (Figure 2).

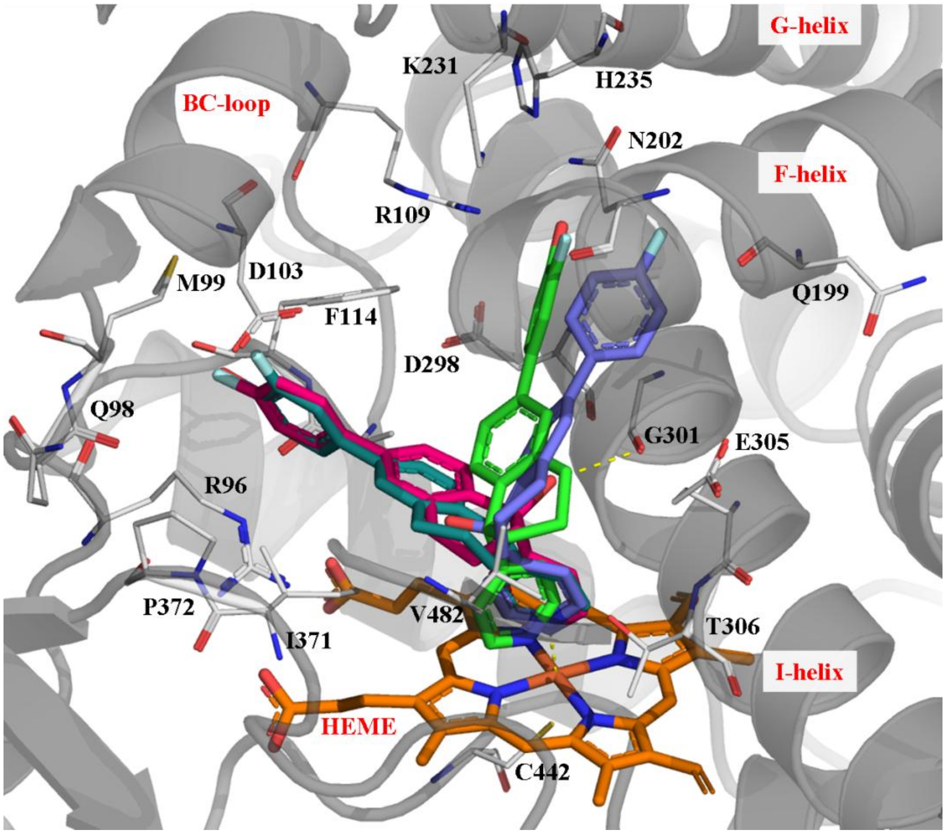

Figure 2. Docking complexes of CYP17 and compounds **5** (R enantiomer in SM1 - bluegreen; S enantiomer in SM2 - violet) and **20** (S enantiomer in SM1 - magenta; R enantiomer in SM2 - green). A dashed yellow line is representing the H-bond between the OH in C17 and the Gly301.

The most important interactions in SM1, besides heme iron complexation, are hydrophobic interactions with Ala113, Phe114, Ile371, Pro372, and Val482 and, depending on substituents R1 and R2, polar ones with the backbone of Glu98 and Met99 and the carboxyl group of Asp103. Furthermore, an H-bond formation between the OH group of R-alkohols and the backbone carbonile of Gly301 was observed, which could act as an additional stabilizing factor. Regarding SM2, the same interactions as described in refs 13e and 13f were found.

Low activity compounds did not show a real clustering in SM1 mode and were characterized by an unsuitable binding angle between the N lone electron pair and the heme plane (compound **12**, Figure 3). On the other hand, the highly active alkenes **13** and **14** bind preferentially in SM2 mode and present a good complexation of the heme iron and a strong binding due to their 3,4-di-OH or 3-F,4-OH substitution.

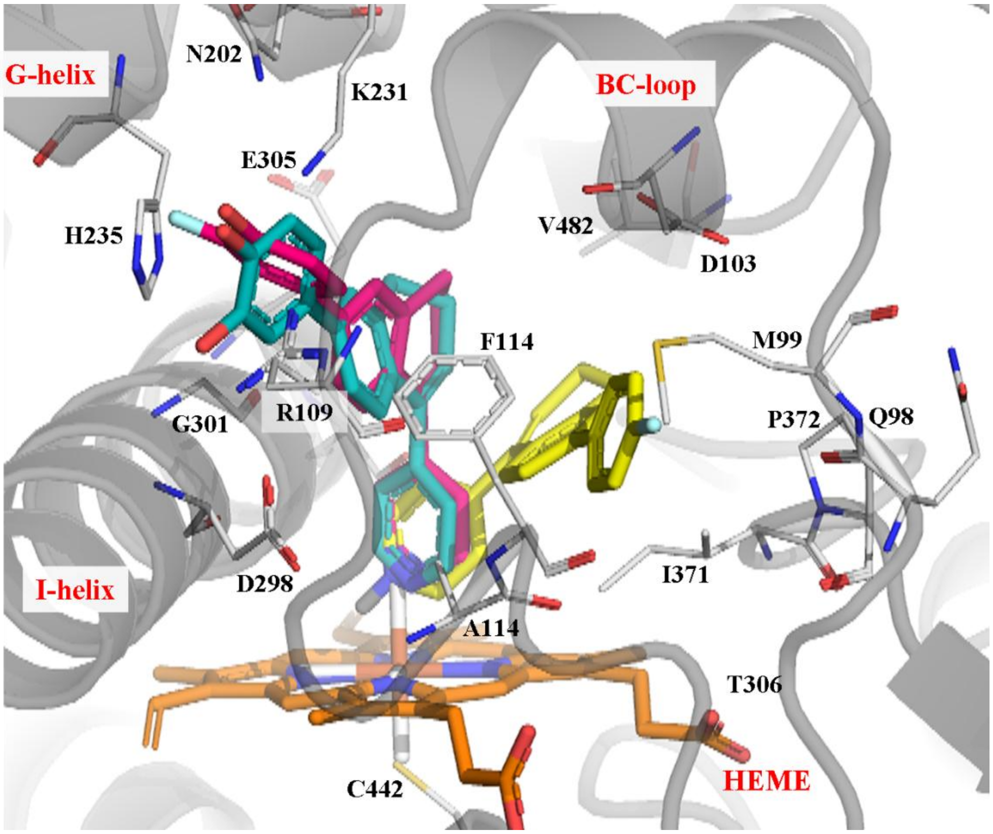

Figure 3. Docking complexes of CYP17 and compounds **11** (yellow; its lone electron pair at the nitrogen and the axis of the octahedral heme iron atom are shown), **13** (cyan) and **14** (magenta).

Discussion and Conclusion

In the class of the indanes the 3-pyridyl substituted unsaturated compound **8**, which is analogous to abiraterone, showed inhibitory activity in contrast to the respective alcohols and alkanes. In the case of the corresponding 4-pyridyl substituted compounds, it is the other way around: the alcohols and the alkanes were active, while the unsaturated compounds did not show activity. However, it became apparent that the five-membered ring, though analogous to the substrate and abiraterone, is not favorable for CYP17 inhibition. On the other hand, the expansion of this ring by one methylene group resulting in the hydronaphthalenes led to very potent and selective compounds. Summarizing, we have discovered nine very potent abiraterone analogues, four of them (**14**, **17**, **19**, and **20**) showing an IC_{50} value below 200 nM and one of them (**20**, IC_{50} = 64 nM) being slightly more active than abiraterone. These compounds showed low inhibition of the CYP enzymes considered for selectivity issues CYP11B1, CYP11B2 and the hepatic CYP3A4. Owing to their promising *in vitro* profile, presently compounds **20** and **19** are separated into their enantiomers and extensively evaluated for their *in vivo* activities and pharmacokinetics in the rat.

Experimental Section

Chemistry. 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. 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).

Method A: Suzuki-Coupling. 5-Bromoindan-1-one (10 mmol) for compounds **1**-**4** or **5b** (10 mmol) for compounds **5**-**7** and the corresponding boronic acid (15 mmol) were dissolved in toluene (70 mL) and Na_2CO_3 (30mL, 2.0 M, aq). The mixture was deoxygenated under reduced pressure and flushed with N_2. After repeating this 3 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 2 times with EtOAc. Then the combined organic extracts were dried over Na_2SO_4, and concentrated under reduced pressure. The purification was performed by Si-gel flash-chromatography.

5-(4-Fluorophenyl)-2,3-dihydroinden-1-one (1a). Synthesized from 5-Bromoindan-1-one and 4-fluorophenylboronic acid according to Method A; yield = 95 %; δ_H ($CDCl_3$, 500 MHz) 2.66-2.69 (m, 2H), 3.12-3.14 (m, 2H), 7.09 (t, *J* = 8.7 Hz, 2H), 7.48 (d, *J* = 8.0 Hz, 1H), 7.53 (dd, *J* = 5.3 Hz, *J* = 8.7 Hz, 2H), 7.56 (s, 1H), 7.75 (d, *J* = 8.0 Hz, 1H); δ_C ($CDCl_3$, 125 MHz) 14.08, 22.63, 25.86, 26.97, 31.57, 36.50, 49.43, 115.84, 116.01, 124.17, 125.02, 126.62, 129.11, 129.17, 136.01, 146.64, 155.86, 162.07, 164.05, 206.40.

5-(4-Methoxyphenyl)-2,3-dihydroinden-1-one (3a). Synthesized from 5-Bromoindan-1-one and 4-methoxyphenylboronic acid according to Method A; yield = 42 %. Since the so obtained ketone was used directly for the nucleophilic addition no analytics was performed.

5-(3-Fluoro-4-methoxyphenyl)-2,3-dihydroinden-1-one (4a). Synthesized from 5-Bromoindan-1-one and 3-fluoro-4-methoxyphenylboronic acid according to Method A; yield = 99 %; δ_H ($CDCl_3$, 500 MHz) 2.64-2.67 (m, 2H), 3.11 (t, *J* = 5.7 Hz, 2H), 3.87 (s, 3H), 6.97 (t, *J* = 8.7 Hz, 1H), 7.27-7.32 (m, 2H), 7.46 (d, *J* = 8.7 Hz, 1H), 7.53 (s, 1H), 7.71 (d, *J* = 8.0 Hz, 1H); δ_C ($CDCl_3$, 125 MHz) 25.85, 36.50, 56.35, 113.71, 115.01, 115.20, 123.20, 123.23, 124.17, 124.58, 126.24, 128.43, 128.55, 131.91, 131.94, 132.04, 132.14, 133.15, 133.22, 135.91, 146.03, 147.90, 148.01, 151.37, 153.82, 155.93, 206.40.

6-(4-Fluorophenyl)-3,4-dihydronaphthalen-1(*2H*)-one (5a). Synthesized from **5b** and 4-fluorophenylboronic acid according to Method A; yield = 89 %; δ_H ($CDCl_3$, 500 MHz) 2.05-2.10 (m, 2H), 2.59 (t, *J* = 6.8 Hz, 2H), 2.92 (t, *J* = 6.1 Hz, 2H), 7.05 (t, *J* = 8.7 Hz, 1H), 7.32 (s, 1H), 7.38 (dd, *J* = 1.7 Hz, *J* = 8.2 Hz, 1H), 7.48 (dd, *J* = 5.3 Hz, *J* = 8.8 Hz, 1H), 8.00 (d, *J* = 8.1 Hz, 1H); δ_C ($CDCl_3$, 125 MHz) 23.29, 29.91, 39.14, 115.76, 115.93, 125.33, 127.12, 127.90, 128.89, 128.96, 131.46, 136.13, 144.92, 145.01, 161.98, 163.95, 198.00.

6-(3,4-Difluorophenyl)-3,4-dihydronaphthalen-1(*2H*)-one (6a). Synthesized from **5b** and 3,4-difluorophenylboronic acid according to Method A; yield = 87 %. Since the so obtained ketone was used directly for the nucleophilic addition no analytics was performed.

6-(3-Fluoro-4-methoxyphenyl)-3,4-dihydronaphthalen-1(*2H*)-one (7a). Synthesized from **5b** and 3-fluoro-4-methoxyphenylboronic acid according to Method A; yield = 59 %; δ_H ($CDCl_3$, 500 MHz) 2.07-2.12 (m, 2H), 2.60 (t, *J* = 6.3 Hz, 2H), 2.94 (t, *J* = 6.1 Hz, 2H), 3.86 (s, 3H), 6.96 (t, *J* = 8.7 Hz, 1H), 7.27-7.30 (m, 2H), 7.33 (s, 1H), 7.39 (dd, *J* = 1.6 Hz, *J* = 8.2 Hz, 1H), 8.00 (d, *J* = 8.2 Hz, 1H); δ_C ($CDCl_3$, 125 MHz) 23.28, 29.92, 39.14, 56.33, 113.62, 114.82, 114.98, 123.01, 124.97, 126.70, 127.94, 131.37, 132.98, 133.03, 144.39, 145.04, 147.80, 147.88, 151.59, 153.55, 198.01.

6-(3,4-Dimethoxyphenyl)-3,4-dihydronaphthalen-1(*2H*)-one (13b). Synthesized from **5b** and 3,4-dimethoxyphenylboronic acid according to Method A; yield = 87 %. Since the so obtained ketone was used directly for the nucleophilic addition no analytics was performed.

Method B: Nucleophilic Addition of the Heterocycle. 3-Iodopyridine (4 mmol) – or either 4-Bromopyridine (4 mmol) for the 4-pyridyl compounds, after basic extraction of its hydrochloride salt with Et_2O (20 mL) and $NaHCO_3$ (satd, aq), followed by drying over Na_2SO_4 and concentration under reduced pressure – was prepared in Et_2O and THF (3:2, 50 mL) at -78°C. Then *n*-BuLi in hexanes (1.6 N, 4.4 mmol) was added and after 5 sec the corresponding ketone (4 mmol) in THF (20 mL) was added at once and the mixture was left stirring for additional 3 h at room temperature. After neutralization with NH_4Cl (satd, aq), the phases were separated, the aqueous layer extracted 2 times with EtOAc and the combined organic extracts dried over Na_2SO_4 and concentrated under reduced pressure. The purification was performed by Si-gel flash-chromatography.

5-(4-Fluorophenyl)-1-(pyridin-3-yl)-2,3-dihydro-*1H*-inden-1-ol (1). Synthesized from **1a** and 3-Iodopyridine according to Method B; yield = 55 %; IR (ATR) υ (cm^{-1}) 2925, 1604, 1516, 1482, 1418, 1223, 1159, 1060, 1027, 822, 713, 685, 605, 537, 507; δ_H ($CDCl_3$, 500 MHz) 2.36-2.47 (m, 2H), 2.85-2.89 (m, 1H), 3.08-3.14 (m, 1H), 6.98-7.04 (m, 3H), 7.14-7.16 (m, 1H), 7.24-7.26 (d, *J* = 7.9 Hz, 1H), 7.36 (s, 1H), 7.41-7.44 (m, 2H), 7.69 (d, *J* = 7.9 Hz, 1H), 8.29 (s, 1H), 8.48 (s, 1H); δ_C ($CDCl_3$, 125 MHz) 14.19, 21.03, 29.89, 44.96, 60.41, 83.55, 115.57, 115.74, 123.07, 123.69, 124.34, 126.30, 128.44, 128.54, 128.73, 128.79, 132.03, 132.11, 133.88, 137.15, 141.02, 142.31, 144.74, 146.32, 147.32, 147.77, 161.54, 163.50, 171.20; *m/z* = 305.17 [M^++H].

5-(4-Fluorophenyl)-1-(pyridin-4-yl)-2,3-dihydro-*1H*-inden-1-ol (2). Synthesized from **2a** and 4-Bromopyridine according to Method B; yield = 50 %;IR (ATR) υ (cm^{-1}) 2931, 1601, 1515, 1482, 1412, 1223, 1159, 1066, 822, 676, 601, 533, 518; δ_H ($CDCl_3$ + CD_3OD, 500 MHz) 2.37-2.47 (m, 2H), 2.92-2.98 (m, 1H), 3.15-3.21 (m, 1H), 7.00 (d, *J* = 7.9 Hz, 1H), 7.04 (t, *J* = 8.6 Hz, 2H), 7.29-7.31 (m, 3H), 7.41 (s, 1H), 7.41-7.47 (m, 2H), 8.38 (d, *J* = 4.8 Hz, 2H); δ_C ($CDCl_3$ + CD_3OD, 125 MHz) 29.90, 44.32, 83.82, 115.44, 115.61, 121.16, 123.46, 124.31, 126.17, 128.63, 128.69, 137.05, 140.95, 144.75, 145.90, 148.70, 156.88, 161.46, 163.42; *m/z* = 306.21 [M^++H].

5-(4-Methoxyphenyl)-1-(pyridin-4-yl)-2,3-dihydro-*1H*-inden-1-ol (3). Synthesized from **3a** and 4-Bromopyridine according to Method B; yield = 45 %; IR (ATR) υ (cm^{-1}) 3165, 2957, 2837, 1606, 1519, 1482, 1456, 1287, 1249, 1182, 1113, 1069, 1039, 1022, 1002, 815, 671, 510, 542; δ_H ($CDCl_3$ + CD_3OD, 500 MHz) 2.34-2.46 (m, 2H), 2.91-2.97 (m, 1H), 3.12-3.20 (m, 1H), 3.77 (s, 3H), 6.90 (d, *J* = 8.8 Hz, 2H), 6.98 (d, *J* = 7.9 Hz, 1H), 7.30-7.32 (m, 3H), 7.42 (s, 1H), 7.44 (d, *J* = 8.8 Hz, 2H), 8.38 (d, *J* = 6.2 Hz, 2H); δ_C ($CDCl_3$ + CD_3OD, 125 MHz) 29.95, 44.43, 55.29, 83.93, 114.19, 121.15, 123.15, 124.20, 125.93, 128.16, 133.49, 141.61, 144.69, 145.29, 148.81, 156.77, 159.17; *m/z* = 318.18 [M^++H].

5-(3-Fluoro-4-methoxyphenyl)-1-(pyridin-4-yl)-2,3-dihydro-*1H*-inden-1-ol (4). Synthesized from **4a** and 4-Bromopyridine according to Method B; yield = 56 %; δ_H ($CDCl_3$, 500 MHz) 2.39-2.48 (m, 2H), 2.93-2.98 (m, 1H), 3.14-3.19 (m, 1H), 3.85 (s, 3H), 6.93-6.98 (m, 2H), 7.21-7.30 (m, 5H), 7.40 (s, 1H), 8.40 (d, *J* = 6.2 Hz, 2H); δ_C ($CDCl_3$, 125 MHz) 30.03, 44.69, 56.36, 84.27, 113.65, 114.81, 114.96, 120.92, 122.74, 123.37, 124.30, 126.11, 134.09, 134.14, 140.74, 144.92, 145.99, 147.16, 147.24, 149.23, 151.57, 153.53, 155.83; *m/z* = 336.11 [M^++H].

6-(4-Fluorophenyl)-1-(pyridin-4-yl)-1,2,3,4-tetrahydronaphthalen-1-ol (5). Synthesized from **5a** and 4-Bromopyridine according to Method B; yield = 44 %; δ_H ($CDCl_3$ + CD_3OD, 500 MHz) 1.75-1.82 (m, 1H), 2.00-2.08 (m, 2H), 2.09-2.16 (m, 1H), 2.86-3.00 (m, 2H), 6.98 (d, *J* = 8.1 Hz, 1H), 7.07 (t, *J* = 8.8 Hz, 2H), 7.27 (dd, *J* = 2.0 Hz, *J* = 8.1 Hz, 1H), 7.33-7.34 (m, 3H), 7.51 (dd, *J* = 5.4 Hz, *J* = 8.6 Hz, 2H), 8.40 (d, *J* = 6.3 Hz, 2H); δ_C ($CDCl_3$ + CD_3OD, 125 MHz) 23.05, 33.68, 44.68, 77.96, 119.19, 119.40, 126.04, 128.82, 131.06, 132.35, 132.43, 133.59, 140.68, 140.71, 141.91, 143.24, 143.34, 152.16, 153.76. 165.19, 167.64; *m/z* = 320.06 [M^++H].

6-(3,4-Difluorophenyl)-1-(pyridin-4-yl)-1,2,3,4-tetrahydronaphthalen-1-ol (6). Synthesized from **6a** and 4-Bromopyridine according to Method B; yield = 40 %; IR (ATR) υ (cm^{-1}) 3063, 1603, 1568, 1525, 1492, 1411, 1320, 1272, 1230, 1198, 1119, 1068, 986, 907, 874, 816, 773, 734, 671; δ_H ($CDCl_3$ + CD_3OD, 500 MHz) 1.81-1.89 (m, 1H), 1.99-2.07 (m, 2H), 2.12-2.18 (m, 1H), 2.89-3.00 (m, 2H), 6.99 (d, *J* = 8.1 Hz, 1H), 7.19 (q, *J* = 8.4 Hz, 1H), 7.24-7.26 (m, 2H), 7.30-7.37 (m, 4H), 8.48 (d, *J* = 6.0 Hz, 2H); δ_C ($CDCl_3$ + CD_3OD, 125 MHz) 20.77, 31.35, 42.36, 117.31, 117.45, 118.95, 119.09, 123.19, 124.35, 124.38, 124.40, 124.43, 126.74, 128.95, 131.14, 139.75, 140.18, 141.49, 150.36, 159.83, 192.24; *m/z* = 338.13 [M^++H].

6-(3-Fluoro-4-methoxyphenyl)-1-(pyridin-4-yl)-1,2,3,4-tetrahydronaphthalen-1-ol (7). Synthesized from **7a** and 4-Bromopyridine according to Method B; yield = 64 %; δ_H ($CDCl_3$ + CD_3OD, 500 MHz) 1.70-1.77 (m, 1H), 1.94-2.03 (m, 2H), 2.03-2.10 (m, 1H), 2.82-2.93 (m, 2H), 3.84 (s, 3H), 6.92 (dd, *J* = 2.7 Hz, *J* = 8.1 Hz, 2H), 6.96 (dt, *J* = 2.4 Hz, *J* = 8.4 Hz, 1H), 7.19-7.28 (m, 6H), 8.35-8.36 (m, 2H); δ_C ($CDCl_3$ + CD_3OD, 125 MHz) 19.02, 29.67, 40.64, 56.06, 73.93, 113.57, 114.23, 114.42, 121.91, 122.37, 122.40, 124.55, 126.74, 129.50, 133.67, 133.73, 137.88, 138.78, 139.11, 146.84, 146.94, 148.20, 151.14, 153.58, 159.42; *m/z* = 350.13 [M^++H].

6-(3,4-Dimethoxyphenyl)-1-(pyridin-4-yl)-1,2,3,4-tetrahydronaphthalen-1-ol (13a). Synthesized from **13b** and 4-Bromopyridine according to Method B; yield = 48 %. Since the so obtained alcohol was used directly for the condensation no analytics was performed.

Method C: Condensation with HCl. The corresponding alcohol (1 mmol) was refluxed in HCl in *i*-PrOH (10 mL, 3N) for 2 h. Afterwards, the resulting solution was concentrated under reduced pressure and washed 3 times with Et_2O. No further purification was necessary.

3-(5-(4-Fluorophenyl)-*3H*-inden-1-yl)pyridine hydrochloride (8). Synthesized from **1** according to Method C; yield = 96 %; IR (ATR) υ (cm^{-1}) 3051, 1601, 1514, 1468, 1229, 1189, 1161, 1104, 1027, 976, 940, 879, 821, 772, 716, 678, 557, 521; δ_H ($CDCl_3$, 500 MHz) 3.53 (s, 2H), 6.61-6.62 (m, 1H), 7.06 (t, *J* = 8.7 Hz, 2H), 7.32 (dd, *J* = 4.8 Hz, *J* = 7.8 Hz, 1H), 7.44 (dd, *J* = 1.4 Hz, *J* = 7.8, 1H), 7.49-7.52 (m, 3H), 7.65 (s, 1H), 7.83-7.85 (m, 1H), 8.55-8.56 (d, *J* = 4.0 Hz, 1H), 8.81 (s, 1H); δ_C ($CDCl_3$, 125 MHz) 38.54, 115.56, 115.73, 120.16, 123.06, 123.50, 125.46, 128.70, 128.76, 131.75, 132.81, 134.83, 137.51, 137.59, 141.72, 142.47, 145.37, 148.79, 148.91, 161.41, 163.37; MS (ESI): *m/z* = 288.13 [M^++H].

4-(5-(4-Fluorophenyl)-*3H*-inden-1-yl)pyridine hydrochloride (9). Synthesized from **2** according to Method C; yield = 100 %; IR (ATR) υ (cm^{-1}) 3482, 3414, 3100-2500, 2075, 1997, 1632, 1602, 1679, 1555, 1469, 1383, 1262, 1221, 1190, 1157, 1097, 1029, 948, 882, 829, 819, 787, 562, 552, 527; δ_H ($CDCl_3$, 500 MHz) 3.20-3.22 (m, 2H), 7.08 (t, *J* = 8.8 Hz, 2H), 7.30 (s, 1H), 7.54 (dd, *J* = 1.7 Hz, *J* = 8.1 Hz, 1H), 7.58 (dd, *J* = 5.4 Hz, *J* = 8.8 Hz, 2H), 7.69 (d, *J* = 8.1 Hz, 1H), 7.76 (d, *J* = 1.4 Hz, 1H), 8.30 (d, *J* = 6.8 Hz, 2H), 8.81 (d, *J* = 6.8 Hz, 2H); δ_C ($CDCl_3$, 125 MHz) 116.53, 116.71, 121.29, 124.36, 126.43, 126.75, 129.90, 129.96, 138.56, 139.74, 141.01, 141.67, 142.35, 142.96, 146.93, 155.08, 162.98, 164.93; *m/z* = 287.96 [M^++H].

4-(5-(4-Methoxyphenyl)-*3H*-inden-1-yl)pyridine hydrochloride (10). Synthesized from **3** according to Method C; yield = 99 %; IR (ATR) υ (cm^{-1}) 3404, 3061, 1632, 1605, 1517, 1496, 1469, 1244, 1185, 1036, 1020, 815, 768, 566; δ_H ($CDCl_3$ + CD_3OD, 500 MHz) 3.24-3.27 (m, 2H), 3.78 (s, 3H), 6.92 (d, *J* = 8.8 Hz, 2H), 7.43 (s, 1H), 7.50 (d, *J* = 8.8 Hz, 2H), 7.53 (dd, *J* = 1.7 Hz, *J* = 8.1 Hz, 1H), 7.60 (d, *J* = 8.1 Hz, 1H), 7.73 (d, *J* = 1.7 Hz, 1H), 8.23 (d, *J* = 6.7 Hz, 2H), 8.80 (d, *J* = 6.7 Hz, 2H); δ_C ($CDCl_3$ + CD_3OD, 125 MHz) 54.88, 114.03, 119.55, 122.91, 124.95, 125.20, 127.89, 133.02, 138.59, 139.21, 140.25, 140.92, 141.10, 144.94, 153.90, 159.08; *m/z* = 301.16 [M^++H].

4-(6-(4-Fluorophenyl)-3,4-dihydronaphthalen-1-yl)pyridine hydrochloride (11). Synthesized from **4** according to Method C; yield = 100 %; IR (ATR) υ (cm^{-1}) 3047, 2536, 1634, 1601, 1489, 1228, 1160, 825, 754, 733; δ_H ($CDCl_3$, 500 MHz) 2.58-2.61 (m, 2H), 2.94 (t, *J* = 7.8 Hz, 2H), 6.55 (t, *J* = 4.7 Hz, 1H), 6.95 (d, *J* = 7.9 Hz, 1H), 7.14 (t, *J* = 8.7 Hz, 2H), 7.38 (d, *J* = 7.9 Hz, 1H), 7.46 (s, 1H), 7.56 (dd, *J* = 5.3 Hz, *J* = 8.7 Hz, 2H), 7.93 (d, *J* = 5.6 Hz, 2H), 8.79 (d, *J* = 5.6 Hz, 2H); δ_C ($CDCl_3$, 125 MHz) 23.92, 27.76, 115.72, 115.90, 124.88, 125.37, 125.91, 127.01, 128.50, 128.56, 130.58, 135.87, 136.21, 137.39, 140.65, 140.67, 158.45, 161.71, 163.68, 190.74; *m/z* = 302.10 [M^++H].

4-(6-(3,4-Difluorophenyl)-3,4-dihydronaphthalen-1-yl)pyridine hydrochloride (12). Synthesized from **5** according to Method C; yield = 98 %;IR (ATR) υ (cm^{-1}) 3067, 1634, 1604, 1522, 1493, 1276, 1200, 1119, 911, 815, 773, 729, 614; δ_H ($CDCl_3$, 500 MHz) 2.57-2.61 (m, 2H), 2.94 (t, *J* = 8.0 Hz, 2H), 6.57 (t, *J* = 4.8 Hz, 1H), 6.96 (d, *J* = 8.0 Hz, 2H), 7.23 (q, *J* = 8.5 Hz, 1H), 7.29-7.31 (m, 1H), 7.36 (d, *J* = 8.5 Hz, 1H), 7.37-7.41 (m, 1H), 7.44 (s, 1H), 7.92 (s, 1H), 7.93 (s, 1H), 8.81 (s, 1H), 8.82 (s, 1H); δ_C ($CDCl_3$, 125 MHz) 23.86, 27.71, 115.77, 115.91, 117.63, 117.76, 122.83, 122.85, 122.87, 122.90, 124.97, 125.35, 125.91, 126.94, 131.12, 136.10, 136.14, 137.54, 139.48, 140.73, 149.11, 149.21, 149.52, 149.62, 151.10, 151.20, 151.50, 151.60, 158.26, 190.74; *m/z* = 320.11 [M^++H].

Method D: Hydration with Pearlman's catalyst. Pearlman's catalyst (10 mass %) and the corresponding alkene was prepared in EtOH and THF (2:1, 5mL) under H_2 atmosphere. The mixture was left stirring for 3 h, then the catalyst was filtered off 3 times and the solution concentrated under reduced pressure. The obtained solid was washed 3 times with Et_2O. No further purification was necessary.

3-(5-(4-Fluorophenyl)-2,3-dihydro-*1H*-inden-1-yl)pyridine hydrochloride (15). Synthesized from **8** according to Method D; yield = 99 %; δ_H ($CDCl_3$, 500 MHz) 1.92-1.98 (m, 1H), 2.65-2.71 (m, 1H), 2.95-3.06 (m, 2H), 4.51 (s, 1H), 6.84 (d, *J* = 6.9 Hz, 1H), 7.00 (t, *J* = 8.6 Hz, 2H), 7.24 (d, *J* = 6.9 Hz, 1H), 7.38-7.41 (m, 3H), 7.76 (s, 1H), 8.13 (d, *J* = 5.0 Hz, 1H), 8.49 (s, 1H), 8.55 (s, 1H); δ_C ($CDCl_3$, 125 MHz) 31.60, 36.55, 48.36, 115.64, 115.66, 115.83, 115.87, 123.89, 123.91, 124.83, 126.54, 126.56, 128.78, 128.84, 140.48, 144.91, 145.03; *m/z* = 290.10 [M^++H].

4-(5-(4-Fluorophenyl)-2,3-dihydro-*1H*-inden-1-yl)pyridine hydrochloride (16). Synthesized from **9** according to Method D; yield = 100 %;IR (ATR) υ (cm^{-1}) 3220, 2927, 2855, 1700, 1621, 1578, 1507, 1439, 1349, 1251, 1215, 1153, 1040, 1027, 986, 914, 831, 806, 784, 748, 699, 668; δ_H ($CDCl_3$, 500 MHz) 2.03-2.09 (m, 1H), 2.72-2.78 (m, 1H), 3.03-3.13 (m, 2H), 4.60 (s, 1H), 6.93 (d, *J* = 7.1 Hz, 1H), 7.06 (t, *J* = 8.6 Hz, 2H), 7.32 (d, *J* = 6.7 Hz, 1H), 7.45-7.47 (m, 3H), 7.68 (s, 2H), 8.71 (s, 2H); δ_C ($CDCl_3$, 125 MHz) 31.80, 36.19, 51.29, 115.66, 115.83, 123.80, 125.02, 126.28, 126.45, 128.72, 128.78, 136.83, 140.75, 140.99, 141.34, 145.22, 161.61, 166.50; *m/z* = 290.17 [M^++H].

4-(6-(4-Fluorophenyl)-1,2,3,4-tetrahydronaphthalen-1-yl)pyridine hydrochloride (17). Synthesized from **11** according to Method D; yield = 97 %; IR (ATR) υ (cm^{-1}) 3049, 1634, 1605, 1518, 1492, 1223, 1160, 1098, 997, 911, 823, 727, 642; δ_H ($CDCl_3$, 500 MHz) 1.82-1.90 (m, 3H), 2.33-2.37 (m, 1H), 2.94-3.00 (m, 2H), 4.46 (s, 1H), 6.78 (d, *J* = 7.5 Hz, 1H), 7.12 (t, *J* = 8.6 Hz, 2H), 7.30 (d, *J* = 6.8 Hz, 1H), 7.39 (s, 1H), 7.53 (dd, *J* = 5.3 Hz, *J* = 8.6 Hz, 2H), 7.67 (s, 2H), 8.71 (s, 2H); δ_C ($CDCl_3$, 125 MHz) 20.06, 29.23, 32.58, 45.49, 115.54, 115.71, 125.26, 126.85, 128.21, 128.43, 128.49, 130.20, 133.17, 136.25, 136.28, 138.09, 139.61, 140.45, 161.51, 163.48, 168.11, 190.64; *m/z* = 304.10 [M^++H].

4-(6-(3,4-Difluorophenyl)-1,2,3,4-tetrahydronaphthalen-1-yl)pyridine hydrochloride (18). Synthesized from **12** according to Method D; yield = 98 %; IR (ATR) υ (cm^{-1}) 3392, 1634, 1604, 1522, 1494, 1269, 1197, 1119, 998, 925, 909, 875, 811, 772, 726, 643; δ_H ($CDCl_3$, 500 MHz) 1.82-1.90 (m, 3H), 2.33-2.37 (m, 1H), 2.94-3.00 (m, 2H), 4.46 (s, 1H), 6.79 (d, *J* = 6.2 Hz, 1H), 7.22 (q, *J* = 8.4 Hz, 2H), 7.26-7.28 (m, 2H), 7.34-7.38 (m, 2H), 7.66 (s, 2H), 8.72 (s, 2H); δ_C ($CDCl_3$, 125 MHz) 20.12, 29.31, 32.64, 45.58, 115.82, 115.96, 117.55, 117.69, 122.86, 122.88, 125.28, 126.94, 128.29, 130.45, 133.90, 138.39, 138.61, 140.64, 167.99, 190.74; *m/z* = 322.11 [M^++H].

4-(5-(Pyridin-4-yl)-5,6,7,8-tetrahydronaphthalen-2-yl)benzene-1,2-diol hydrobromide (19). Synthesized from **13** according to Method D; yield = 98 %;IR (ATR) υ (cm^{-1}) 3172, 2930, 1634, 1603, 1524, 1495, 1446, 1263, 1196, 1114, 805, 634; δ_H ($CDCl_3$ + CD_3OD, 500 MHz) 1.73-1.80 (m, 3H), 2.19-2.24 (m, 1H), 2.81-2.89 (m, 2H), 4.27-4.32 (m, 1H), 6.63-6.65 (m, 1H), 6.81 (d, *J* = 8.2 Hz, 1H), 6.88-6.90 (m, 1H), 7.00 (s, 1H), 7.17 (d, *J* = 8.0 Hz, 1H), 7.28 (s, 1H), 7.45-7.51 (m, 2H), 8.59 (s, 2H); δ_C ($CDCl_3$ + CD_3OD, 125 MHz) 20.18, 29.33, 32.56, 45.39, 113.74, 115.36, 118.77, 124.72, 126.50, 127.66, 129.98, 137.81, 144.22, 144.65; *m/z* = 318.65 [M^++H].

2-Fluoro-4-(5-(pyridin-4-yl)-5,6,7,8-tetrahydronaphthalen-2-yl)phenol hydrobromide (20). Synthesized from **14** according to Method D; yield = 96 %; IR (ATR) υ (cm^{-1}) 3066, 1635, 1607, 1525, 1494, 1366, 1293, 1215, 1117, 911, 872, 812, 780, 727, 644; δ_H ($CDCl_3$ + CD_3OD, 500 MHz)

1.84-1.92 (m, 3H), 2.34-2.40 (m, 1H), 2.92-3.00 (m, 2H), 4.48-4.50 (m, 1H), 6.76 (d, *J* = 8.0 Hz, 1H), 7.02 (t, *J* = 8.8 Hz, 1H), 7.22 (d, *J* = 8.4 Hz, 1H), 7.26-7.28 (m, 2H), 7.38 (s, 1H), 7.73 (s, 1H), 7.74 (s, 1H), 8.79 (s, 1H), 8.80 (s, 1H); δ_C ($CDCl_3$ + CD_3OD, 125 MHz) 24.03, 33.17, 36.48, 49.51, 118.12, 118.27, 121.90, 126.73, 128.77, 131.08, 131.69, 134.12, 136.34, 136.39, 136.79, 142.06, 143.29, 144.45, 148.04, 148.15, 154.59, 156.50, 173.21; *m/z* = 320.08 [M^++H].

Method E: Ether Cleavage and Condensation with HBr. The corresponding ether was refluxed overnight in HBr (100 mL for compound **5c**, 3 mL for compounds **13** and **14**). For compound **5c**, after reaction completion water (100 mL) was added, the mixture was cooled down and filtered. The solid was washed 3 times with water and no further purification was needed. For compounds **13** and **14**, after reaction completion the mixture was neutralized with solid $NaHCO_3$ and extracted 3 times with EtOAc. The combined organic extracts were dried over Na_2SO_4, concentrated under reduced pressure and purified by Si-gel flash-chromatography.

6-Hydroxy-1-tetralone (5c). Synthesized from 6-methoxy-1-tetralone according to Method E; yield = 89 %; δ_H ($CDCl_3$, 500 MHz) 2.01-2.06 (m, 2H), 2.56 (t, *J* = 6.8 Hz, 2H), 2.83 (t, *J* = 6.1 Hz, 2H), 6.66 (d, *J* = 6.1 Hz, 1H), 6.74 (dd, *J* = 2.4 Hz, *J* = 8.6 Hz, 1H), 7.38 (s, 1H), 7.91 (d, *J* = 8.6 Hz, 1H); δ_C ($CDCl_3$, 125 MHz) 23.27, 29.92, 38.80, 114.60, 114.66, 125.75, 130.20, 147.82, 161.28, 198.69.

4-(5-(Pyridin-4-yl)-7,8-dihydronaphthalen-2-yl)benzene-1,2-diol hydrobromide (13). Synthesized from **13a** according to Method E; yield = 65 %; IR (ATR) υ (cm^{-1}) 3127, 1634, 1605, 1533, 1503, 1396, 1315, 1252, 1205, 1123, 806, 788, 752, 617; δ_H ($CDCl_3$ + CD_3OD, 500 MHz) 2.53-2.55 (m, 2H), 2.87 (t, *J* = 7.8 Hz, 2H), 6.53-6.55 (m, 1H), 6.83-6.89 (m, 2H), 6.94-6.96 (m, 1H), 7.28-7.31 (m, 1H), 7.40-7.41 (m, 1H), 7.94-7.96 (m, 2H), 8.77-8.79 (m, 2H); δ_C ($CDCl_3$ + CD_3OD, 125 MHz) 27.23, 31.01, 117.13, 118.87, 122.01, 127.96, 128.02, 128.97, 129.45, 129.78, 131.43, 132.96, 135.65, 139.43, 139.55, 140.53, 144.17, 144.88, 148.02, 148.27, 162.74; *m/z* = 316.18 [M^++H].

2-Fluoro-4-(5-(pyridin-4-yl)-7,8-dihydronaphthalen-2-yl)phenol hydrobromide (14). Synthesized from **7** according to Method E; yield = 28 %; IR (ATR) υ (cm^{-1}) 3385, 1605, 1522, 1489, 1437, 1416, 1313, 1291, 1278, 1221, 1199, 1168, 117, 1067, 1008, 909, 891, 874, 823, 806778, 735, 667, 621; δ_H ($CDCl_3$ + CD_3OD, 500 MHz) 2.49-2.53 (m, 2H), 2.94 (t, *J* = 7.9 Hz, 2H), 6.25-6.27 (m, 1H), 7.02 (q, *J* = 8.4 Hz, 2H), 7.26 (d, *J* = 8.1 Hz, 1H), 7.31 (s, 1H), 7.34 (d, *J* = 5.8 Hz, 1H), 7.39-7.42 (m, 3H), 8.58 (d, *J* = 5.7 Hz, 2H); δ_C ($CDCl_3$ + CD_3OD, 125 MHz) 24.90, 29.47, 50.22, 50.37, 50.39, 50.55, 50.57, 115.51, 115.66, 117.44, 119.29, 124.08, 124.10, 125.27, 125.75, 126.63, 127.40, 131.57, 133.51, 134.13, 134.18, 138.60, 1388.77, 140.54, 145.40, 145.52, 150.25 150.87, 152.09; *m/z* = 318.08 [M^++H].

1-Tetralon-6-yl trifluoromethanesulfonate (5b). 6-Hydroxy-1-tetralone (5 g, 30.83 mmol) was prepared with pyridine (2.77 mL, 33.91 mmol) in dry DCM (100 mL) at 0°C. After carefully adding trifluoromethanesulfonic anhydride (5.10 g, 30.83 mmol) over 1 min, the reaction mixture was left stirring for 3 h at room temperature. Afterwards, excess trifluoromethanesulfonic anhydride was neutralized and the crude was washed 2 times with water, the organic phase dried over Na_2SO_4 and concentrated under reduced pressure. Further purification was performed by Si-gel flash-chromatography; yield = 30 %; δ_H ($CDCl_3$, 500 MHz) 2.08-2.14 (m, 2H), 2.61 (t, *J* = 6.8 Hz, 2H), 2.95 (t, *J* = 6.1 Hz, 2H), 7.12-7.14 (m, 2H), 8.06 (d, *J* = 8.4 Hz, 1H); δ_C ($CDCl_3$, 125 MHz) 22.90, 29.73, 38.77, 119.68, 119.94, 121.36, 130.04, 132.39, 147.11, 152.34, 196.40.

Biological Assays. CYP17 Preparation and Assay. As source of human CYP17, our *E. coli* system[19] (coexpressing human CYP17 and NADPH-P450 reductase) was used and the assay was performed as previously described[12a] using unlabeled progesterone as substrate and applying HPLC with UV-detection for separation.

Inhibition of CYP11B1 and CYP11B2. V79MZh11B1 or V79MZh11B2[22] cells expressing the respective human enzyme were used and our assay procedure using [4-^{14}C]-11-deoxycorticosterone was performed.[20]

Inhibition of CYP3A4. The recombinantly expressed enzymes from baculovirus-infected insect microsomes and cytochrome b5 (BD Supersomes™) were used and the manufacturer's instructions (www.gentest.com) were followed.

Molecular Modeling. Various inhibitors of Tables 1 were docked into our CYP17 homology model by means of the GOLD v3.2 software[23] using GOLDSCORE and CHEMSCORE. 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.

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[24] as implemented in Sybyl.

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-hybridized nitrogen of the pyridine and the iron of the heme was set. Additionally, the goldscore.p450_pdb parameters were used and 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.[23] 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[25] and the representative structure of each significant cluster was selected. After the docking simulations and cluster analysis were performed, the quality of the docked representative poses was evaluated based on visual inspection of the putative binding modes of the ligands. The latter were further evaluated by means of Silver v3.1.1, the postprocessing tool of GOLD, and at last by GOLDSCORE.

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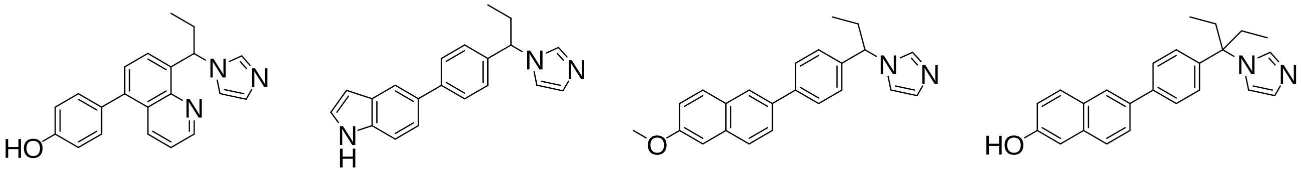
VI. Results

VI.1. Overview

VI.1.a. CYP17 Inhibitors. Annulations of Additional Rings in Methylene Imidazole Substituted Biphenyls: Synthesis, Biological Evaluation and Molecular Modelling

In our aim to explore the spatial limitations surrounding both areals corresponding to the substrates A- and C-ring, two main classes of differently A- and C-ring annulated compounds were synthesized. The C-ring annulated compounds (11 compounds) showed no to moderate inhibition of CYP17 at 2 µM with exception of compound **III.9** (Figure 11), which showed 71 % inhibition, much higher than the reference compound ketoconazole (19%). The A-ring annulated compounds (10 compounds) presented much better activities, and the most potent ones, compounds **III.13** (75%), **III.19** (75%) and **III.21** (74%) showed comparable ones to compound **III.9**.



compound **III.9** compound **III.13** compound **III.19** compound **III.21**

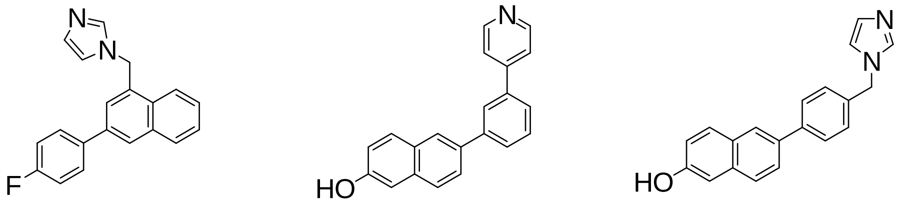
Figure 11. Most active compounds of the A- (**III.**13, **III.**19 and **III.**21) and C-ring (**III.**9) annulated classes.

Regarding selectivity over other CYP enzymes, all compounds exhibited inhibition, higher than 85% at 2 µM except from **III.21** (32% at 2 µM and 64% at 10 µM). **III.9** and **III.21**, most active compounds of each substance class, were subsequently tested on CYP11B1, showing both of them similarly strong inhibitions of 86 and 94% at 0.2 and 2 µM for compound **III.9**, and 81 and 95% at the same concentrations for **III.21**.

Based on our biological results and molecular modeling studies of this and previous work, it is striking that the correct spatial extension in both areals is favorable for both activity and selectivity. The importance of the presence of an ethyl group at the methylene linker for achieving higher potencies was reiterated.

VI.1.b. Synthesis, Biological Evaluation and Molecular Modelling Studies of Novel ACD- and ABD-Ring Steroidomimetics as Inhibitors of CYP17

Since we observed good results at the expansion of both A- and C-ring mimicking benzenes, we expanded both with a rigid core to the corresponding ABD- and ACD-ring mimetics. Thus, we synthesized 11 ACD- and 6 ABD-ring mimetics and again, the expansion at the A-ring was overall more favorable for activity than at the C-ring. The 3- and 4-pyridyl substituted ACD-ring mimetics were slightly active, the 4-pyridyls minimally better, while the methylene-imidazolyl ones were moderately active, among them compound **IV.9** (Figure 12) showed a CYP17 inhibition of 50% at 2 µM. The same relationship was observed for the ABD-ring mimetics, yielding the 4-pyridyl **IV.15** with 66% and the methylene-imidazolyl **IV.17** with 61% inhibition, both measured at 2 µM.



compound **IV.9** compound **IV.15** compound **IV.17**

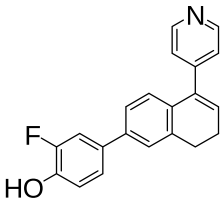
Figure 12. Most active compounds of the ACD- (**IV.9**) and ABD-ring (**III.15** and **IV.17**) mimetics classes.

As for selectivity against CYP3A4, only compound **IV.15** showed low inhibition at 1 µM (6%) and 10 µM (19%). In contrast to the A- and C-ring annealed compounds, **IV.15** exhibited very low activities of 3 and 23% at 0.2 and 2 µM respectively on CYP11B1.

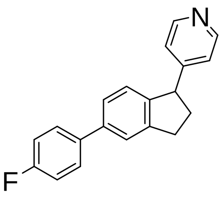
Again, good inhibition values were achieved by occupying the areals surrounding the steroidal A- and C-rings, as observed in our enzyme model and through appropriate substitution, selectivity over other CYP enzymes was shown. The rigidity attained through mimicking the D-ring with a benzene showed good results, similar to the rigidity gained through the ethyl group at the methylene linker in other substance classes.

VI.1.c. Synthesis, Biological Evaluation, and Molecular Modeling of Abiraterone Analogues: Novel CYP17 Inhibitors for the Treatment of Prostate Cancer

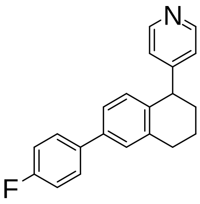
Through the knowledge gained so far about the spatial limitations and geometry of a good CYP17 inhibitor, as well as of the binding site, we went on to replace the D-ring by a nonaromatic ring, in some way a hybrid between our ethyl-substituted (olefinic) and naphthalenic (aromatic) compounds. In such way, we obtained nonsteroidal 3-pyridyl substituted abiraterone mimetics, which showed inactive, and other 4-pyridyl indane derivatives (altogether 9 indane derivatives were prepared), which showed none to moderate CYP17 inhibition, except for compound **V.16** (IC_{50} = 233 nM, Figure 13). Another synthesized class was the class of hydronaphthalenes (11 compounds), in which the synthesized alcohols showed none to moderate activities, the alkenes exhibited a very wide range of activities (the most potent one: 4-hydroxyl compound **V.14**: IC_{50} = 188 nM), and the alkanes proved the most potent ones, being the 4-hydroxyl compounds **V.19** (IC_{50} = 144 nM) and **V.20** (IC_{50} = 64 nM) the most active ones together with the 4-F **V.17** (IC_{50} = 163 nM). 11, out of the 20 synthesized compounds, were more active than ketoconazole (IC_{50} = 2780 nM), and **V.20** even slightly better than abiraterone (IC_{50} = 72 nM) and also highly active in the whole cell assay (IC_{50} < 200 nM).



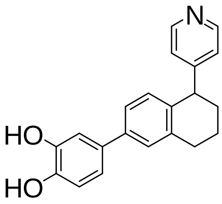
compound **V.14**



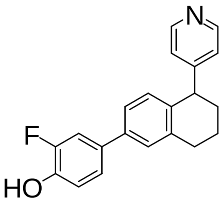
compound **V.16**



compound **V.17**



compound **V.19**



compound **V.20**

Figure 13. Most active compounds of the indane (**V.16**) and hydronaphthalene (**V.14**, **V.17**, **V.19** and **V.20**) classes.

These five most potent compounds proved much better selectivities over CYP3A4, CYP11B1 and CYP11B2 than ketoconazole. Compared to abiraterone, they were all comparable to it regarding CYP3A4 (IC_{50} = 2704 nM), with the exception of **V.16** and **V.19** with higher inhibitions, and **V.20** with a lower one (IC_{50} = 3386 nM). They all showed comparable or even higher selectivities regarding CYP11B1 than abiraterone (IC_{50} = 1608 nM), but regarding CYP11B2, only **V.14** and **V.19** showed respectively slightly higher and lower inhibitions, while most of them were much less selective, all around 500 nM, compared to abiraterone (1751 nM). This makes compounds **V.19** and **V.20** respectively potent and selective CYP17, and dual CYP17 and CYP11B2 inhibitors.

After docking of both substrates, abiraterone, and our biphenyls and abiraterone analogues into our enzyme model, two separate binding modes were observed. The substrates show more poses in one of both binding modes, thus they seem to bind rather in that one, which is the same binding mode preferred by the most potent compounds, both biphenyls and abiraterone analogues. Good polar interactions are contemplated between the 4-hydroxy groups and the backbone of Glu98 and Met99 and the carboxyl group of Asp103.

VI.2. Summary

In the class of the annulated compounds, two sub-classes were synthesized: A-ring annulated and C-ring annulated compounds. In the first class, one highly active compound was found (71% inhibition at 2 µM), while in the second one three showed around 75% inhibition. Only **III.21**, belonging to the C-ring annulated class, was selective over CYP3A4 and none of them over CYP11B1.

Among the classes of the ACD- and ABD-ring mimetics, one moderate active compound with 50% inhibition was found for the ACD-mimetics, while in the other class 2 compounds with inhibitions around 65% were discovered. Only **IV.15**, an ABD-mimetic, was selective against both CYP3A4 and CYP11B1.

The abiraterone analogues were divided in 5- and 6-membered ring analogues, and additionally in alcohol, alkene, and alkane classes. The most potent compounds were 6-membered ring alkanes; four of them were highly active, one of which was good (**V.19**) and another one very good selective (**V.20**) against both CYP3A4 and CYP11B1, and furthermore **V.19** was additionally selective against CYP11B2.

Close to half of the presented compounds were more active and selective than the off-label drug ketoconazole, while regarding abiraterone acetate, a drug undergoing clinical trials, four exhibited comparable or even better activities, and two of them showed good selectivities for CYP11B1 and CYP3A4, while one of the latter was also highly selective against CYP11B2.

The importance of our three main pharmacophores was present throughout the work. The complexation by the sp^2 nitrogen with the heme iron, the rigidity of the core structure, especially in the D-ring areal and the polar interactions between the substituents mimicking the substrate's 3-hydroxy group always contributed to better activities, and sometimes even to selectivities. OH and F substituents were overall optimal, while the moiety and substitution pattern of the sp^2 nitrogen bearing aromatic ring varies according to the nature of the core structure.

VII. Discussion and Conclusion

VII.1. Relevance of this Work

The motivation for this work was the absence of effective mild treatments for prostate cancer, disease which affects a large sector of elder male population. The purpose of our research was to find a new medical therapy for the treatment of prostate cancer which suits the real necessities of the patients, based on recent scientific discoveries. Tumor growth is stimulated by androgens, produced by a series of enzymatic steps, in which CYP17 takes a key role for the biosynthesis of all of them. Other, androgen suppressing therapies have been widely used in the past, proving effective therapeutic results in the treatment of prostate cancer, leading in many cases to tumor shrinkage and eradication, but created heart problems and promoted the development of the disease, and were then unable to treat the mutated tumor. Recent discoveries show that through inhibition of CYP17 tumor growth may be successfully stopped in all prostatic adenocarcinomas at all stages. These discoveries acquaint CYP17 not only as the most promising current target for the treatment of prostate cancer, but also as a self-sufficient future therapy option. Ketoconazole is the only CYP17 inhibitor which was used off-label for the treatment of prostate cancer, which had low activity and no selectivity toward other CYP enzymes, but still showed significant clinical improvements. Several other research groups are investigating in this field, leading to one promising steroidal drug, abiraterone acetate, currently undergoing clinical studies.

Nonsteroidal and selective drugs have the advantage over the steroidal ones of having strongly reduced side effects. Such endocrine and intracrine drugs have furthermore a comfortable form of administration, as they can be taken orally. Thus, nonsteroidal medicines which inhibit CYP17 selectively represent a mild, safe and efficient treatment option for prostate cancer which is even more crucial in the handling of prostate cancer patients, since the large majority is of advanced age. All this makes successfully designed nonsteroidal CYP17 inhibitors optimal medicines for the treatment of prostate cancer.

The *in vivo* suppression of plasma testosterone levels in rats has been proven several times by us through the administration of compounds belonging to some of these classes, pointing to an accomplished proof of concept. In such manner, after the appropriate, still outstanding trials, some of the presented lead compounds would be optimal candidates for future clinical studies, aiming the approval of novel, innovative drugs for the treatment of prostatic adenocarcinoma for any stage of disease, while some others could be ideal hit compounds for the development of other such drugs. For the latter compounds, proposals for the improvement of both activity and selectivities were explained for each class based on the biological activities, and our enzyme model and diverse data available for our and other CYP enzymes.

VII.2. Achievements and Future Perspectives

Through our medicinal chemistry approaches, we were successful in finding several highly active and selective lead compounds in three different classes, and to further develop the concept of inhibitors of CYP17, essential for a safe therapy for the kind of patients affected by prostate cancer, patients in advanced age. Altogether we present four highly active compounds with activities similar or better than abiraterone (72 nM) in our enzymatic test. One of them is not only more active, but also much more selective than abiraterone regarding CYP11B1 and CYP11B2, and only slightly more active at CYP3A4.

The organic compounds found, suitable as new drugs for the treatment of prostate cancer through inhibition of CYP17, are still to undergo many other preclinical studies after enantiomeric separation, such as other *in vitro* cell-free and whole-cell assays of different enzymes, and *in vivo* tests in different animal species with a high enzyme homology to the human phenotype, such as rats, dogs and monkeys. Just after the successful completion of such tests, clinical tests may be started.

The results of this work also serve as a very good starting point for future new generation CYP17 inhibitors, regarding the functionalities (A- and C-ring substitutions, core rigidity, heme-complexing nitrogen) described before found to lead to good activities and selectivities in most of the investigated classes.

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