Non-steroidal CYP Enzyme Inhibitors as Potential Treatments for Corticoid and Estrogen Related Diseases

Dissertation

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von

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You have to believe in yourself. That's the secret of success. (Charles Chaplin, American actor)

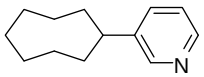
ABSTRACT

Steroidogenic CYP enzymes are responsible for the biosynthesis of steroid hormones, which regulate vital physiological processes. Pathologically elevated levels of these hormones are closely related to severe hormone dependent diseases. Therefore, down-regulations of these hormone levels by inhibition of the corresponding synthase are promising pharmacotherapies.

Abnormally high concentrations of aldosterone are associated with severe cardiovascular diseases such as congestive heart failure, hypertension, and myocardial fibrosis. Since CYP11B2 is the crucial enzyme in the biosynthesis of aldosterone, the inhibition of CYP11B2 is proposed as an innovative treatment for the related diseases. Although our previously designed naphthalene pyridine type of CYP11B2 inhibitors showed strong potency and good selectivity toward CYP11B1, notable inhibition toward hepatic enzyme CYP1A2 was observed. In order to improve the selectivity toward CYP1A2, modifications were performed on the core structure by altering cycle size and presenting H-bond forming groups and / or bridge bonds leading to a series of 3-pyridinyl substituted aliphatic cycles. It was discovered that dearomatisation of the lipophilic core and destruction of planar configuration significantly reduced CYP1A2 inhibition. Thus, potent and selective CYP11B2 inhibitors, e.g. **I-12**, were obtained. Furthermore, via ligand-based drug design approach a series of N containing heterocycle substituted indolines and indoles were designed and synthesized. It has been found that the bulkiness of amido moiety is detrimental for CYP11B2 inhibition. Accordingly, Me was the most suitable substituent. Electron donating groups on the pyridyl increased the potency, whereas electron withdrawing groups reduced the inhibition. This study yielded highly potent and selective toward CYP11B1 CYP11B2 inhibitors, e.g. **II-20**.

Moreover, breast cancer patients under CYP19 inhibitor therapy are vulnerable to cardiovascular diseases because estrogen deficiency leads to elevated levels of aldosterone and thus to a higher risk. Therefore, dual inhibitors of CYP19 / CYP11B2 are promising therapeutics for breast cancer patients with the benefit of reducing cardiovascular diseases. By combining the key structural features of selective CYP19 and CYP11B2 inhibitors into one molecule, a series of pyridinylmethyl substituted 1,2,5,6-tetrahydro-pyrrolo[3,2,1-*ij*]quinolin-4-ones were designed and synthesized with **III-25** as the most promising CYP19 / CYP11B2 dual inhibitor with good potency and selectivity.

Furthermore, CYP11B1, which is responsible for the biosynthesis of cortisol, is a promising target for the treatment of hypercortisolism (Cushing's syndrome). Starting from etomidate several structure modifications led to compound **IV-4** as a potent CYP11B1 inhibitor.



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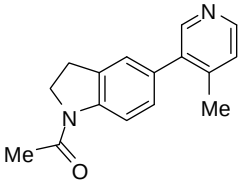

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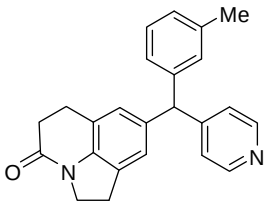

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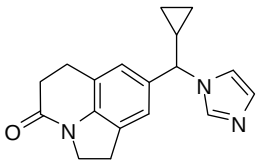


IV-4

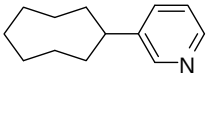
ZUSAMMENFASSUNG

Steroidogenic CYP-Enzyme sind verantwortlich für die Biosynthese von Steroid-Hormonen, die wichtige physiologische Prozesse regulieren. Pathologisch erhöhte Werte dieser Hormone stehen in engem Zusammenhang mit einigen schwerwiegenden hormonabhängigen Krankheiten. Daher bestehen in der Absenkung dieser Hormonspiegel durch Hemmung der entsprechenden Synthase vielversprechende Pharmakotherapien.

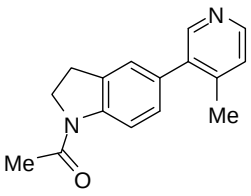
Ungewöhnlich hohe Konzentrationen von Aldosteron sind mit schweren kardiovaskulären Erkrankungen wie Herzinsuffizienz, Hypertonie und myokardialer Fibrose assoziiert. Da CYP11B2 das entscheidende Enzym bei der Biosynthese von Aldosteron ist, wurde die Hemmung von CYP11B2 als vielversprechende Behandlung für die Krankheiten vorgeschlagen. Obwohl unsere Naphthalin Pyridin CYP11B2 Inhibitoren eine starke Aktivität und gute Selektivität gegenüber CYP11B1 zeigten, wiesen sie dennoch eine bemerkenswerte Hemmung gegenüber dem hepatischen CYP1B2 auf. Um die Selektivität zu diesem hepatischen Enzym zu verbessern, wurden Veränderungen an der Kernstruktur durch Veränderung von Zyklus Größe und H-Brücken bildenden Gruppen durchgeführt und so eine Serie von 3-Pyridinyl substituierten aliphatischen Zyklen erhalten. Es wurde gefunden, dass Dearomatisierung des lipophilen Kerns und die Aufhebung der planaren Konfiguration erheblich die CYP1A2-Hemmung reduziert. So wurden potente und selektive CYP11B2 Inhibitoren, z.B. **I-12**, erhalten. Ferner wurden durch Ligand-basiertes Wirkstoff-Design eine Reihe von N heterocyclisch substituierten Indolinen und Indolen synthetisiert. Es wurde festgestellt, dass die Sperrigkeit der Amido-Einheit für die CYP11B2 Hemmung nachteilig ist. Entsprechend ist Me der am besten geeignete Substituent. Elektronen spendende Gruppen am Pyridyl erhöhen die Potenz, während Elektronen ziehende Gruppen die Hemmung reduzieren. Diese Studie führte zu hoch potenten und selektiven CYP11B2 Inhibitoren, z.B. **II-20**.

Brustkrebspatientinnen unter CYP19 Inhibitor Therapie sind anfällig für Herz-Kreislauf-Krankheiten, weil Östrogenmangel zu hohen Aldosteron Konzentrationen führt und damit das Risiko erhöht. Daher sind duale Inhibitoren von CYP19 / CYP11B2 für Brustkrebspatientinnen hochinteressant, da sie kardiovaskuläre Komplikationen verhindern können. Durch Kombination der wichtigsten strukturellen Merkmale von selektiven CYP19 und CYP11B2 Inhibitoren, wurde eine Reihe von Pyridinylmethyl substituierten 1,2,5,6-Tetrahydropyrrolo [3,2,1-ij] chinolin-4-onen entwickelt, z. B. **III-25**.

CYP11B1 ist die verantwortlich für die Biosynthese von Cortisol und damit ein vielversprechendes Target für die Behandlung von Hypercortisolismus (Cushing-Syndrom). Ausgehend von Etomidat wurde durch verschiedene Structurmodifizierungen **IV-4** als potenter CYP11B1 Inhibitor erhalten.



I-12



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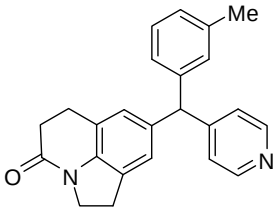
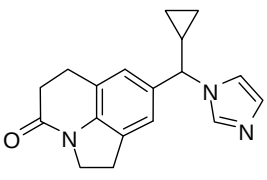


III-25



IV-4

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ABBREVIATIONS

ACTH	adrenocorticotropic hormone
ACE	angiotensin converting enzyme
AI	aromatase inhibitor
Ang II	angiotensin II
AR	androgen receptor
AT_1	angiotensin II receptor type 1
AT_2	angiotensin II receptor type 2
AVP	arginine vasopressin
BC	breast cancer
Boc	*tert*-butoxycarbonyl
CHF	congestive heart failure
CRH	corticotrophin-releasing hormone
CVD	cardiovascular diseases
CYP	cytochrome P450
CYP11A1	P450scc, cholesterol side-chain cleavage enzyme
CYP11B1	11β-hydroxylase
CYP11B2	aldosterone synthase
CYP17	17α-hydroxylase-17,20-lyase
CYP19	aromatase, estrogen synthase
DHEA	dehydroepiandrosterone
DMAP	4-dimethylaminopyridine
DME	dimethyl ether
DMF	dimethylformamide
DOC	11-deoxycorticosteone
EtOAc	ethyl acetate
e.e.	enantiomeric excess
ENaC	epithelial sodium channel
ER	estrogen receptor
ESI	electrospray ionization
ETO	etomidate
FAD	fadrozole
GABA	gammaaminobutyric acid
GnRH	gonadotropin-releasing hormone
GR	glucocorticoid receptor
GREs	glucocorticoid-responsive elements

HSD	hydroxysteroid dehydrogenase
KET	ketoconazole
MET	metyrapone
MF	Myocardial fibrosis
mp	melting point
MR	mineralocorticoid receptor
NBS	*N*-bromosuccinimide
NCI	national cancer institute
n.d.	not determined
NEP	neutral endopeptidase
PCC	pyridinium chlorochromate
PE	petroleum ether
PgR	progesterone receptor
RAAS	renin–angiotensin–aldosterone system
RGS2	regulator of G protein signaling-2
ROS	reactive oxygen species
SARs	structure-activity relationships
SERM	selective estrogen receptor modulator
SF	selectivity factor
SGK1	serum glucocorticoid-regulated kinase
t_R	retention time
TFA	trifluoroacetic acid
THF	tetrahydrofurane
TLC	thin layer chromatography
TMS	trimethylsilyl

CONTENTS

1 Introduction

1.1 Cytochrome P450 Superfamily

1.1.1 General

Cytochrome P450 (abbreviated as CYP) is a super family of isoenzymes containing a cysteine bound protoporphyrin IX as prosthetic group (Figure 1A). The name of CYP was derived from a unique spectroscopic property. When heme iron in the reduced state binds to carbon monoxide, an intense Soret peak at 450 nm is found.[1] Not all heme-containing proteins but CYP enzymes exhibit an absorption peak at 450 nm due to a thiolate anion from the cysteine residue is present as the 5th ligand. CYPs are ubiquitous in nature, and exist in all species from prokaryotes to humans. According to amino acid sequence identity, CYPs are categorized into families and subfamilies. Families share > 40% of sequence identity, whereas subfamilies have > 55% of sequence identity. The members of CYP superfamily continue to increase as more and more genomic sequences are identified. Up to date, more than 9000 genes are identified. For humans, there are 18 families and 44 subfamilies.[2]

For humans and animals, CYP enzymes are membrane bound, and play a wide range of functions in humans through mixed-function oxidations. In order to carry out the oxidative reactions, CYPs require a source for electrons, which is implemented via an electron transfer chain. Two distinct types of electron transfer chains exist according to the location in the cell of the CYP. CYP enzymes (e.g. CYP11B1, CYP11B2) located in the mitochondrial inner membrane follow NADPH → ferredoxin reductase → ferredoxin → CYP to transfer electrons, whereas CYPs located in the endoplasmic reticulum (e.g. CYP17, CYP19) pass electrons through NADPH → NADPH CYP reductase → CYP. In some cases (e.g. CYP17, CYP3A4), cytochrome b5 plays a role in the electron transfer chain.

1.1.2 Oxidative Mechanism of CYP Enzymes

CYPs as monooxidases refer to incorporation of one atom of oxygen molecule into the corresponding substrate and another one atom into water. They can catalyze a diverse range of oxidative reactions, such as hydroxylation of aliphatic or aromatic CH groups, oxidation of hereroatoms, epoxidation of double bonds and dealkylation reactions. The classic catalytic mechanism[3] is depicted in Figure 1. The process of catalytic reaction is divided into several consecutive steps, including substrate binding, 1st electron transfer, oxygen molecule binding, 2nd electron binding, two protonations and release of the product. In the active center, heme iron is located. The catalytic oxidation thus starts from the resting state **1**, in which a molecule of water is coordinated to the iron as the sixth ligand, on the side opposite to the cysteine residue. As the substrate binds in the active site, the water molecule is usually displaced and arrives at complex **2**. Also, the state of the heme complex often varies from low spin to high spin. CYPs inhibitors are able to competitively bind to the active iron and exhibit inhibitory potency for the treatment of some relevant diseases. Accompanying the change of electronic state, an electron is transfered from NAD(P)H to heme complex via electron transfer

chain, resulting in the reduction of the ferric iron to ferrous iron **3**. As follows, an oxygen molecule covalently binds to the heme iron at the distal axial coordination position of the heme center, resulting in an oxy-P450 complex **4**, as the last relatively stable intermediate. A second electron is subsequently transferred to the oxy-P450 complex to form a peroxo-ferric complex **5a**. Afterwards, it is instantly protonated by local transfer from water or from surrounding amino acid residues to form a hydroperoxo-ferric complex **5b**. A second protonation accompanying hemolytic scission of O–O bond and releasing one water molecule results in a highly oxidative iron(IV)-oxo species **6**. Oxygen atom transfer from **6** to the bound substrate leads to the oxygenated product complex **7**. After release of the product, the catalytic cycle completes with a water molecule returning to occupy the distal coordination position. Under physiological conditions, some side reactions can also occur, which causes an abortive result by the return of the hemoenzyme to the state **2**.

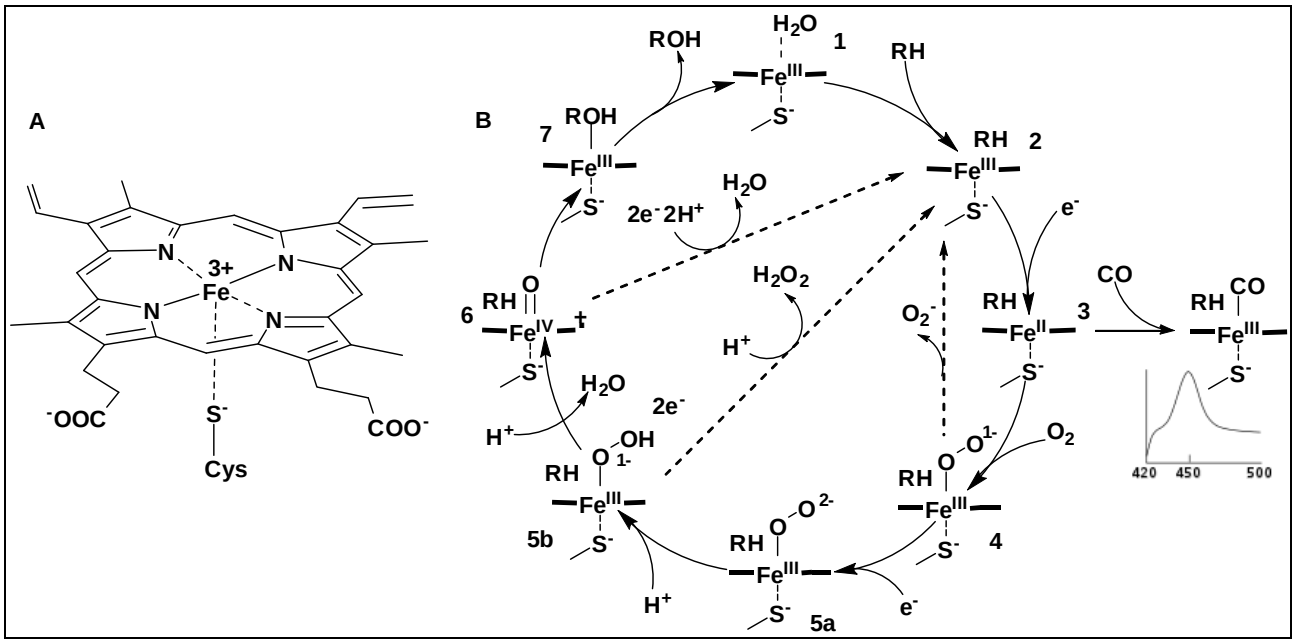


Figure 1. A) CYP cofactor. B) CYP catalytic circle.

1.1.3 Functions of CYP Enzymes

In nature, CYP enzymes play a diverse range of functions. In general, they contribute to the maintenance of homeostasis of endogenous and exogenous substances. In humans and animals, CYP1–3 families are predominantly located in the liver, responsible for the elimination of exogenous chemicals including drugs by providing the molecules with polar groups such as OH, whereas, CYP 4–51 families play an important role in the biosynthesis or deactivation of crucial endogenous substances, which regulate the development and homeostasis of the body, such as steroidal hormones, arachidonic acid, fatty acids, thromboxane, bile acids, prostacyclins and vitamins.

1.1.4 Hepatic CYP Enzymes

As mentioned above, due to the predominant location of CYP1–3 families in the live, they are termed as hepatic CYP enzymes. Another characteristic of these enzymes is that there is a broad range of substrates for each enzyme. This ensures a vast majority of the exogenous chemicals can be metabolized by hepatic CYPs to protect the body from toxic effects. It is estimated that metabolism including phase I and phase II in the

liver accounts for the elimination of approximately 75% of the 200 top prescribed drugs (Figure 2A).[4] In phase I, CYP1–3 families provide the drugs with polar functional groups, mostly OH, which can be further attached to a hydrophilic compound in phase II to form a conjugate in order to facilitate the excretion of the modified drugs as more water soluble forms. There is approximately 75% of metabolism achieved by CYPs. Among them, CYP3A subfamily (predominantly CYP3A4) is responsible for 46% of drug oxidations, whereas CYP2C9 for 16% and CYP2C19 plus CYP2D6 for 12%. The metabolism percentage for each CYP enzyme may be explained by its corresponding expression ratio in the liver (Figure 2B). CYP3A4, the predominant enzyme expressed in the liver, accounts for 34.4% of the total CYP1–3 families, whereas CYP2C9 for 17.3%, and CYP2E1 and CYP1A2 for 14.6% and 12.5% respectively[4,5].

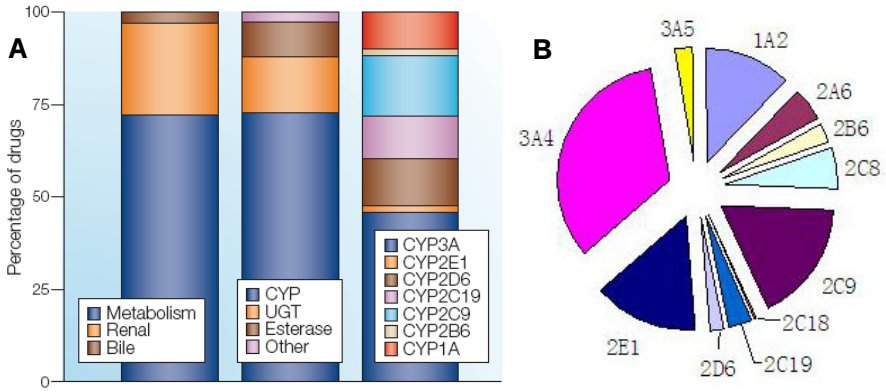


Figure 2. A) Metabolism and elimination of 200 top prescribed drugs. B) Liver expression ratio of CYP enzymes.

Since hepatic CYPs play a vital role in drug metabolism, adverse drug reactions caused by CYPs should be taken into account, e.g. too short half-life, toxic metabolites or unfavorable drug-drug interactions. Sometimes a drug needs to be given together with other drugs which may show inhibition of CYP enzymes (involved in the drug's metabolism). As a result, the drug can be overdosed in the patient, and thus causes side effects or even leads to death. On the other hand, if they induce the activity of CYP enzymes, the drug can decrease levels due to over metabolism and cause ineffectiveness. Therefore, whether or not drug candidates show effects on hepatic CYP enzymes is important to be taken into account before they enter into clinical use in order to decrease attrition rates in drug development. The insight into substrate features or X-ray structures of metabolizing CYPs may allow for the design of compounds with more favorable drug metabolism properties. For example, CYP3A4 oxidizes a wide range of lipophilic compounds, while CYP1A2 prefers relatively small and planar lipophilic molecules. This is due to the fact that in the binding site of CYP1A2 is surrounded by a few aromatic residues.[6] Moreover, a polymorphism refers to a difference in DNA sequence, which is found in at least 1% of a population. This can lead to a different CYP metabolizing activity, resulting in poor metabolizer, normal metabolizer or high metabolizer. This may result in that drug dosage given does not give rise to curative effects in high metabolizers, but is able to cause overdose which results in subsequent toxicity in poor metabolizers. Since CYP1A2, 2D6, 2C9, 2C19 and 3A4 account for over 90% of drug metabolism, generally taking care of them can be very helpful for drug design and development.

1.1.5 Steroidogenic CYP Enzymes

By contrast with hepatic CYP enzymes, steroidogenic CYPs are specific due to one enzyme only has one or a few substrates, which are always steroids. Sterodogenic CYP enzymes include CYP11A1, CYP11B1 (11ß-hydroxylase), CYP11B2 (aldosterone synthase), CYP17 (androgen synthase), CYP19 (estrogen synthase) and CYP21. They are of great importance in the biosynthesis of crucial hormones which are responsible for the regulation of vital physiological processes in the body. Their biosyntheses starting from cholesterol are depicted in Figure 3. Exogenous or endogenous cholesterol undergoes side-chain cleavage catalyzed by CYP11A1 to provide pregnenolone which is transformed to progesterone by 3ß-HSD. Pregnenolone and progesterone thus undergo hydroxylation and lyase reaction, catalyzed by CYP17, to form dehydroepiandrosterone (DHEA) and androstenedione, respectively. They are important precursors of androgens such as androstenediol, testosterone and dihydrostestosterone. In addition, androstenedione and testosterone can be catalyzed by CYP19 to synthesize estrogens, estrone and estradiol, respectively. On the other side, CYP21 oxidizes progesterone and 17α-hydroxyprogesterone to form 11-deoxycorticosteone (DOC) and 11-deoxycortisol, which are further catalyzed by CYP11B2 and CYP11B1 to yield aldosterone (major mineralocorticoid) and cortisol (major glucocorticoid), respectively.

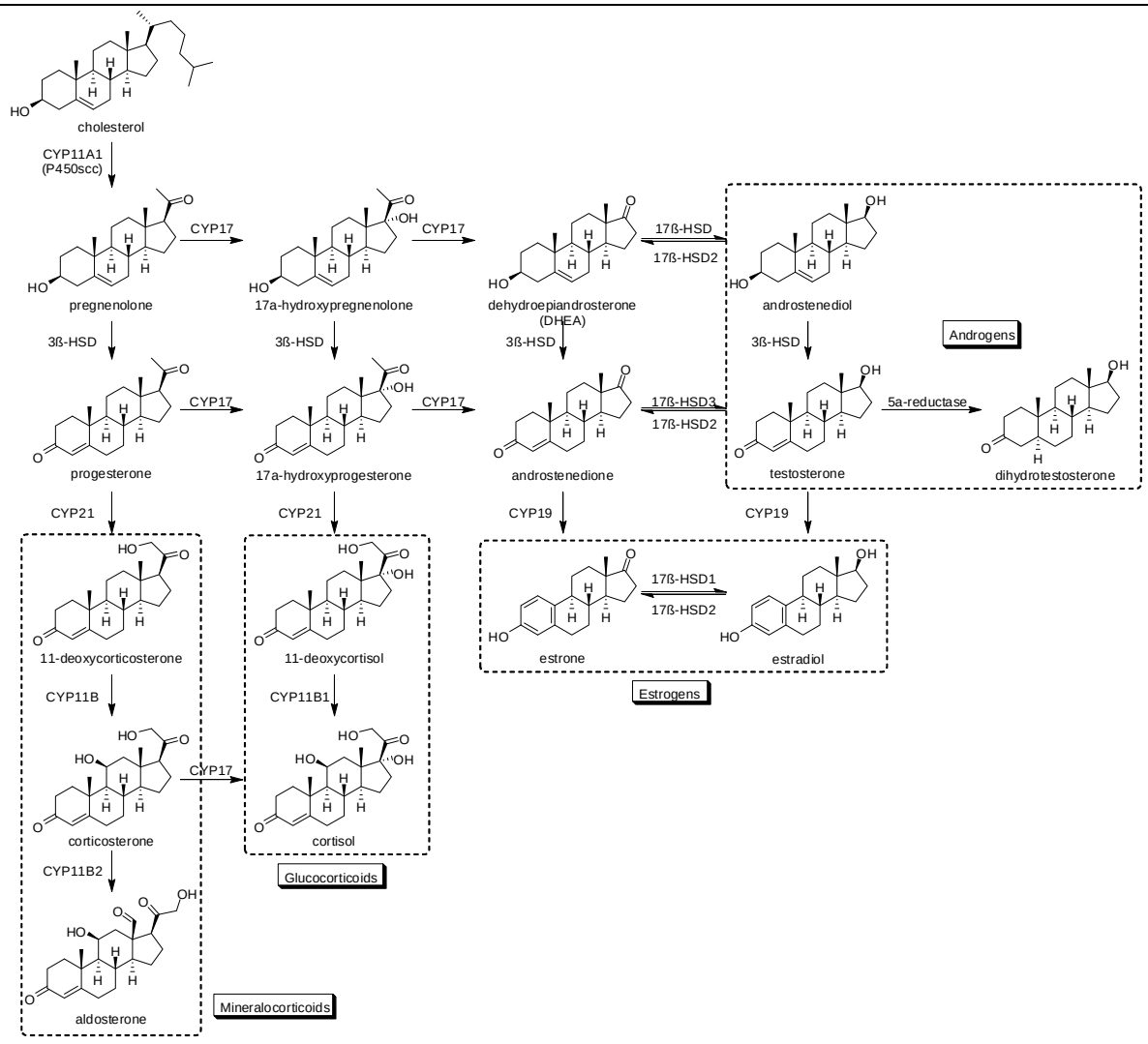


Figure 3. Steroidogenic CYP enzymes in the biosynthesis of steroid hormones.

In the whole biosynthesis route, all CYP enzymes are indispensable for in vivo physiological processes. Their appropriate amount and activity determine whether or not the physiological functions are normal. The majority of them has been employed as pharmaceutical targets for the treatment of the diseases relevant to their overexpression and consequently elevated levels of their products, steroidal hormones. CYP11B1, which is responsible for the synthesis of cortisol, is used as a target for Cushing's syndrome for over five decades. Nevertheless, CYP11B2 inhibitors, as a promising alternative pharmaceutical therapy for severe cardiovascular diseases such as congestive heart failure (CHF) and hypertension associated with high aldosterone concentration are of interest for preclinical development. Besides, CYP17, key enzyme of androgen biosyntheses, is a promising target for the treatment of hormone dependent prostate cancer. Similarly, the inhibition of CYP19, involved in estrogen biosynthesis, The only CYP enzyme which has already been widely implemented in the clinic is CYP19 in patients with postmenopausal breast cancer (BC), as it catalyzes the last step of estrogen biosynthesis.

1.2 CYP11B2 Inhibitors and Cardiovascular Diseases

1.2.1 Aldosterone: Physiology and Pathology

1.2.1.a. Molecular Mechanism of Action and Regulation of Fluid Homeostasis

Aldosterone plays an essential role in the regulation of electrolyte and volume homeostasis. In genomic manner, aldosterone binds to mineralocorticoid receptor (MR), which is a member of nuclear receptor family, in epithelia of the distal tubules and the collecting ducts in kidney. This causes MR/aldosterone complex conformation change in the ligand-binding domain, and induces the dissociation of associated proteins such as heat shock protein. After quick translocation to the nucleus, MR/aldosterone complex binds, as a dimer, to hormone response elements, and regulates the expression of aldosterone-responsive genes like serum glucocorticoid-regulated kinase (SGK1)[7] which is a key aldosterone-responsive gene responsible for the retention of sodium, resulting in stimulation of transepithelial ion transport. The typical transepithelial ion channel is amiloride-sensitive epithelial sodium channel (ENaC) and basolateral Na^+/K^+-ATPase pump, which leads to absorption of sodium and water at the expense of potassium excretion, consequently causing the increase of blood volume and pressure. The latency from binding reaction to final effect normally lasts 2 h – 4 h. Moreover, aldosterone in physiological concentration was found to act in a nongenomic manner to rapidly increase (within minutes) intracellular calcium and pH value in human distal colon via regulation of basolateral K^+ channels.[8]

1.2.1.b. Aldosterone in the Pathological Progression of Hypertension, Myocardial Fibrosis and Congestive Heart Failure

However, numerous studies demonstrated that high plasma levels of aldosterone are associated with the pathogenesis of cardiovascular diseases such as hypertension, myocardial fibrosis and congestive heart failure. After excessive aldosterone binds to MR, a resultant over-expression of sodium channels in the target cells stimulates sodium absorption. The increase in blood pressure causes natriuresis, and the release of natriuretic factors. Thus, this results in the excretion of a lot of sodium and reduces the elevated fluid volume,

in spite of excessive aldosterone. When the plasma volume is restored, blood pressure continues to rise. This correlates with the development of a rise in systemic vascular resistance. Furthermore, abnormally high levels of aldosterone exhibit different adverse effects on the heart[9]. One of the most documented impacts of aldosterone on the heart is myocardial fibrosis which is an important feature of cardiac hypertrophy and left ventricular dysfunction, although the mechanism is not well understood to date. In rat models, treatment with a MR antagonist resulted in a blockage of fibrosis at doses that do not improve the hypertension[10,11]. This indicates that the effect of aldosterone on fibrosis is independent of hemodynamic processes, and may directly effect the deposition of collagens in cardiac fibroblasts[12,13] and the induction of tissue necrosis via a reparative process[14], resulting in worsening the myocardial stiffness. It is well established that in addition to adrenal production of aldosterone, local aldosterone synthesis, e.g. in heart[15], is identified. It is postulated that the extra-adrenal synthesis of aldosterone contributes to pathogenesis of heart diseases. Besides, the fact that MR is found in non-epithelial tissues[16] such as heart and vasculature provides the basis of local effect of aldosterone on the cardiac tissues. Moreover, aldosterone, acting as a potent pro-inflammation factor[17,18], induces reactive oxygen species[19]. This also contributes to vascular fibrosis and endothelium stiffening[20], and may be closely associated with atherosclerosis[21,22]. Accompanying severe myocardial fibrosis, cardiac hypertrophy and adverse structural remodelling of myocardium occur, and lead to diastolic dysfunction. As a consequence, the reduction of cardiac output and renal hyporefusion result in congestive heart failure (CHF). In addition, inflammation, hypertension, reperfusion injury, and myocardial infarction induced by excessive aldosterone contribute to the progression of heart failure[23,24].

1.2.2 Regulation of Aldosterone Secretion by AngII, Potassium and ACTH

It has been shown that aldosterone's essential role in salt and volume homeostasis is controlled by negative feedback loops of renin-angiotesin-aldosterone system (RAAS) and potassium. There are three major stimuli in regulation of aldosterone secretion (Figure 4), including angiotensin II (AngII), potassium and adrenocorticotropic hormone (ACTH).

Renin an aspartyl protease is synthesized in renal juxtaglomerular apparatus. Renin catalyzes circulating angiotensinogen produced in liver to form inactive angiotensin I, which is converted to AngII by angiotensin converting enzyme (ACE) located in multiple tissues. After Ang II binds to its receptor AT_1, aldosterone synthesis is stimulated and the hormone is released from the adrenal cortex. Stimulation factors of renin secretion include renal perfusion pressure, β-adrenergic stimulation and prostaglandins, while potassium and Ang II as well as atrial natriuretic peptide are inhibitory factors.[25] It is noteworthy to mention that elevated Ang II inhibits renin secretion via a small negative feedback loop, independent of blood pressure and aldosterone concentration. Moreover, the amount of aldosterone secreted is strongly stimulated by potassium, which is independent of RAAS. An increase of potassium causes a rise in aldosterone secretion, and the change of aldosterone levels, in turn, depresses the increase of potassium via a small negative feedback loop. In addition, other regulatory factors such as ACTH, endothelin and serotonin[25] are characterized as response to stress. ACTH is a pituitary peptide, acting as the most potent stimulator of acute aldosterone secretion; however, chronic stimulation leads to an unexpected decrease in aldosterone release.

1.2.3 Components of RAAS and Treatment of High Levels of Aldosterone Related Diseases — State of Art

Due to the fact that high aldosterone levels are closely associated with the progression of several cardiovascular diseases as mentioned above, a direct or indirect block aldosterone is applicable to the treatment of the relevant diseases. Since aldosterone is totally involved in the RAAS, it is feasible to find appropriate targets from the components of the system. Depression of aldosterone secretion or inhibition of its function could be such targets. In clinical use, several strategies are implemented, consisting of MR antagonists, ACE inhibitors, AT1 receptor antagonists, and renin inhibitors. In addition, a novel promising approach of direct inhibition of aldosterone production by CYP11B2 inhibitors is still of interest.

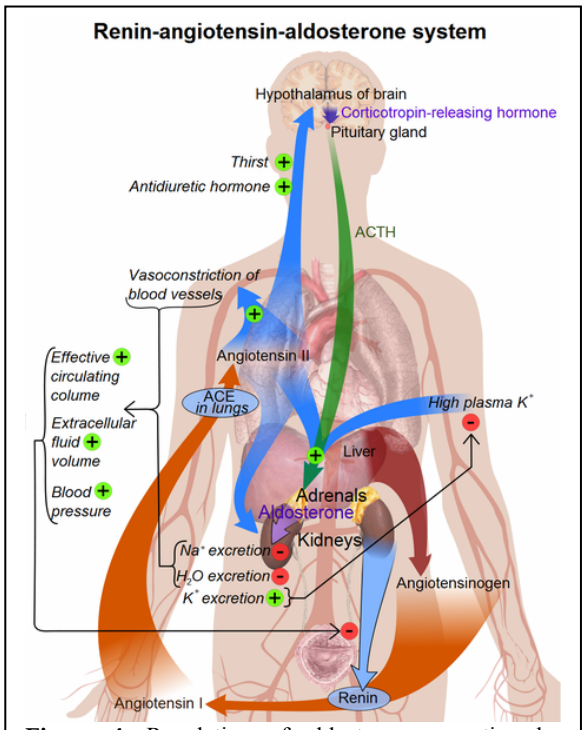


Figure 4. Regulation of aldosterone secretion by RAAS, potassium and ACTH (adoption from http://www.zazzle.com/wordsunwords/gifts?cg=196948684080670328)

1.2.3.a. Mineralocorticoid Receptor Antagonists

To date, only two steroidal MR antagonists are brought to the market for the treatment of hypertension and heart failure, i.e. spironolactone, eplerenone (Figure 5). In recent years, several series of non-steroidal MR antagonists have been designed and patented by pharmaceutical companies, such as imidazole carboxamides[26] from Exelixis and 3,3-bisaryl oxindoles[27] from Lilly (Figure 5). In clinical trails, both spironolactone[28] and eplerenone[29] have been demonstrated that they showed effective in decreasing morbidity and mortality in patients with heart failure. However, severe adverse effects like gynaecomastia and breast pain are found[28], due to poor selectivity of spironolactone toward other members of nuclear receptor family such as androgen receptor (AR) and progesterone receptor (PR). Although the sex hormone

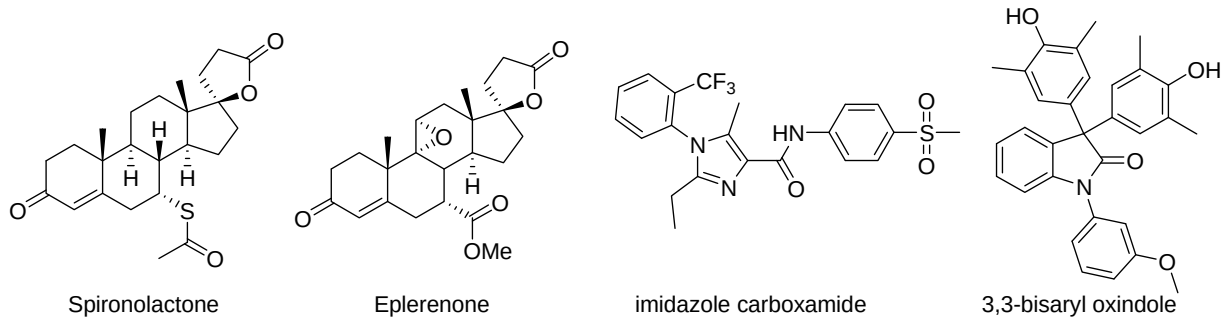


Figure 5. Typical MR antagonists: steroids (spironolactone, eplerenone) and nonsteroids (imidazole carboxamide, 3,3-bisaryl oxindole).

related side effects have been weakened by the more selective steroidal MR antagonists eplerenone, long-term treatment leaves high levels of aldosterone unaffected[30], which can lead to further exacerbation of heart dysfunction in a MR independent non-genomic manner[31] Moreover, both spironolactone and eplerenone can

cause hyperkalemia which further leads to death.

1.2.3.b. ACE Inhibitors

ACE is located in multiple tissues, predominantly in the lung, and is the key enzyme to produce Ang II. The latter stimulates vasoconstriction and aldosterone secretion. Captopril (Figure 6), the first ACE inhibitor, was approved by FDA in 1981 for the treatment of hypertension and CHF. This was regarded as a breakthough due to both the novel mechanism of action and the pioneering in structure-based drug design. Moreover, ACE inhibitors are also used in the treatment of diabetic renal failure to delay the progress of diabetic nephropathy[32]. More recently other non-sulphydryl-containing ACE inhibitors came to the market following enalapril (Figure 6) in order to improve patient compliance. However, ACE inhibitors may cause a persistent cough in common and angioedema[33] associated with elevated bradykinin levels. Besides, long-term inhibitory effects of ACE inhibitors on plasma aldosterone levels are reduced due to the phenomenon of "aldosterone escape"[34,35] via ACE-independent pathways[36].

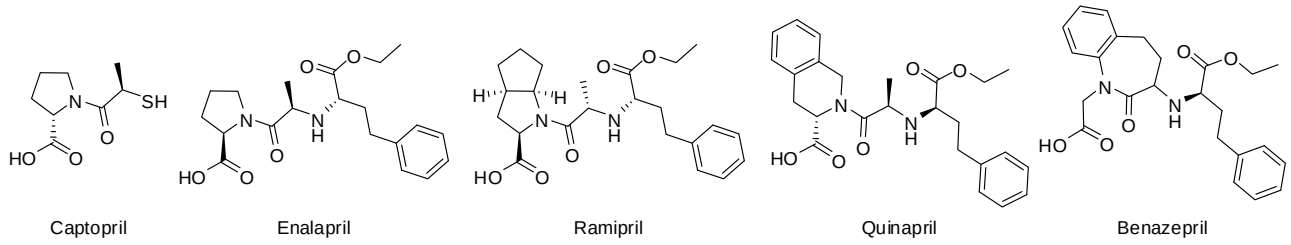


Figure 6. Typical ACE inhibitors.

1.2.3.c. Angiotensin II Receptor antagonists

Since ACE exhibits multiple effects and not all of them are mediated through angiotensin II receptors, therefore, blockage of angiotensin II receptors may result in more specific actions and less side effects than observed with ACE inhibitors. There are various subtypes of angiotensin II receptors, of which both AT_1 and AT_2 are classified to the superfamily of G-protein coupled receptors[37]. Most of the known biologic effects of angiotensin II, including the stimulation of aldosterone secretion, are mediated by AT_1 receptor. In contrast, the AT_2 receptor plays an important role in counteracting some of angiotensin II effects mediated by the AT_1 receptor[38]. Losartan (Figure 7) is the first orally active and specific AT_1 receptor antagonist, which was approved by FDA in 1994 for the treatment of hypertension. In addition, AT_1 receptor antagonists also serve as an approach for diabetic nephropathy and CHF. After losartan, other AT_1 receptor antagonists (Figure 7) with different pharmacokinetic profiles were marketed. Due to a good tolerability profile, AT_1 receptor antagonists are primarily implemented for hypertensive patients, who are intolerant of ACE inhibitor therapy. However, long-term AT_1 receptor antagonist treatment leads to chronically increased levels of Ang II and its biodegradation products, which may produce their own effects in vivo[39]. With the aim of achieving the maximal benefits of blocking the RAAS, the combination of an ACE inhibitor and an AT1 receptor antagonist seems attractive to improve the overall blockade of this system, but the benefits of the combination are still controversial[40].

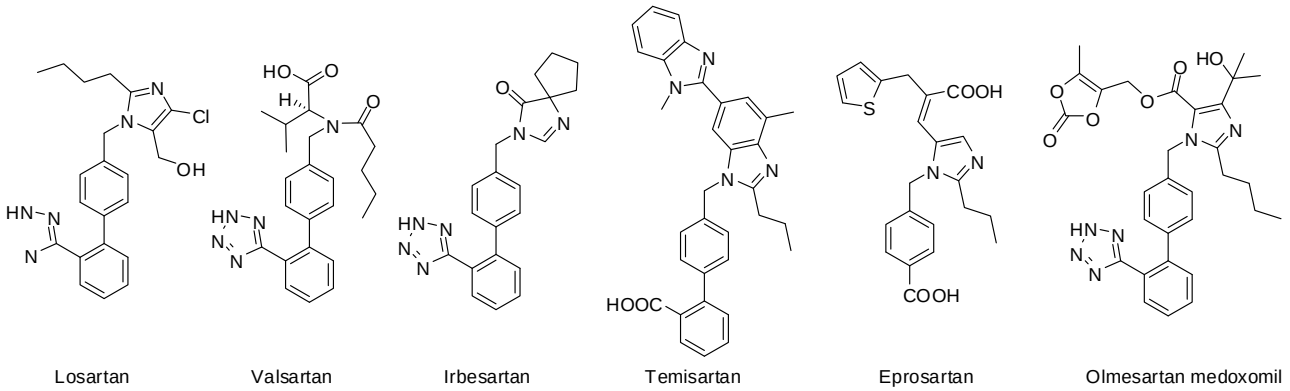


Figure 7. Typical AT_1 receptor antagonists.

1.2.3.d. Renin Inhibitors

Renin plays a pivotal role in the RAAS as the first component, and renin inhibitors are applied for the treatment of hypertension. There are three generations of renin inhibitors: peptide compounds, peptide-like compounds and non-peptide compounds. The first and second generation of renin inhibitors, CPG29287 and CGP38560 (Figure 8) as representative inhibitors respectively, exhibited strong activity in vitro but were discontinued due to immune reaction or lack of oral bioavailability. Optimization of peptide analogue CGP38560 led to the first orally active renin inhibitor, aliskiren (Figure 8), developed by Novartis in cooperation with Speedel and approved in 2007 by FDA as an antihypertensive agent[41]. Over a decade, more renin inhibitors with less chiral centers and less complexity in synthesis were designed, e.g. piperidines[42-45], piperazinones[46,47] (Figure 8).

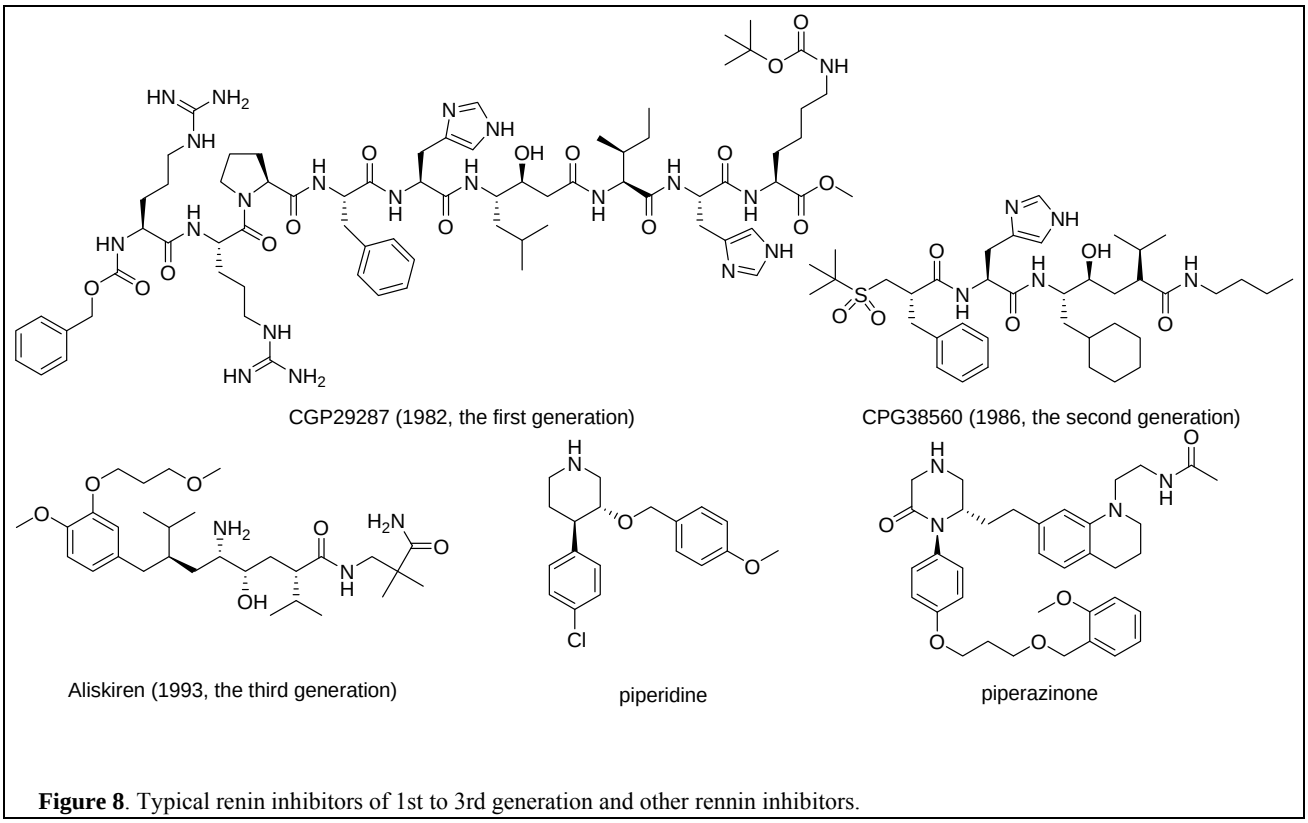


Figure 8. Typical renin inhibitors of 1st to 3rd generation and other rennin inhibitors.

1.2.4 Selective CYP11B2 Inhibitors for the Treatment of Hypertension, Myocardial Fibrosis and Congestive Heart Failure

Aldosterone is the terminal component of RAAS, which is closely related to the pathological progression of some severe cardiovascular diseases such as hypertension, myocardial fibrosis and CHF as mentioned above. Direct reduction of aldosterone biosynthesis by inhibition of aldosterone synthase (CYP11B2), which is primarily located in the zona glomerulosa of the adrenal cortex, is a promising strategy to treat those diseases associated with abnormally high aldosterone levels. This idea was proposed as early as in 1994[48] by our working group. Although to date there is no CYP11B2 inhibitor launched as a drug, recent in vivo studies in rats have demonstrated that CYP11B2 inhibitors can reduce plasma aldosterone levels[49,50]. Long-term administration of FAD286 (*R*-enantiomer of fadrozole, Figure 9) to rats with heart failure improves cardiac haemodynamics and cardiac function, which is more significant than those by MR antagonist, spironolactone[51]. With respect to the design of CYP11B2 inhibitors, in addition to potency, selectivity towards other important steroidal CYPs such as CYP11B1, CYP17, and CYP19 should also be emphasized since selectivity is closely associated with severe side effects. Especially selectivity toward CYP11B1 is a challenging issue due to the high homology (over 93%)[52] between CYP11B1 and CYP11B2.

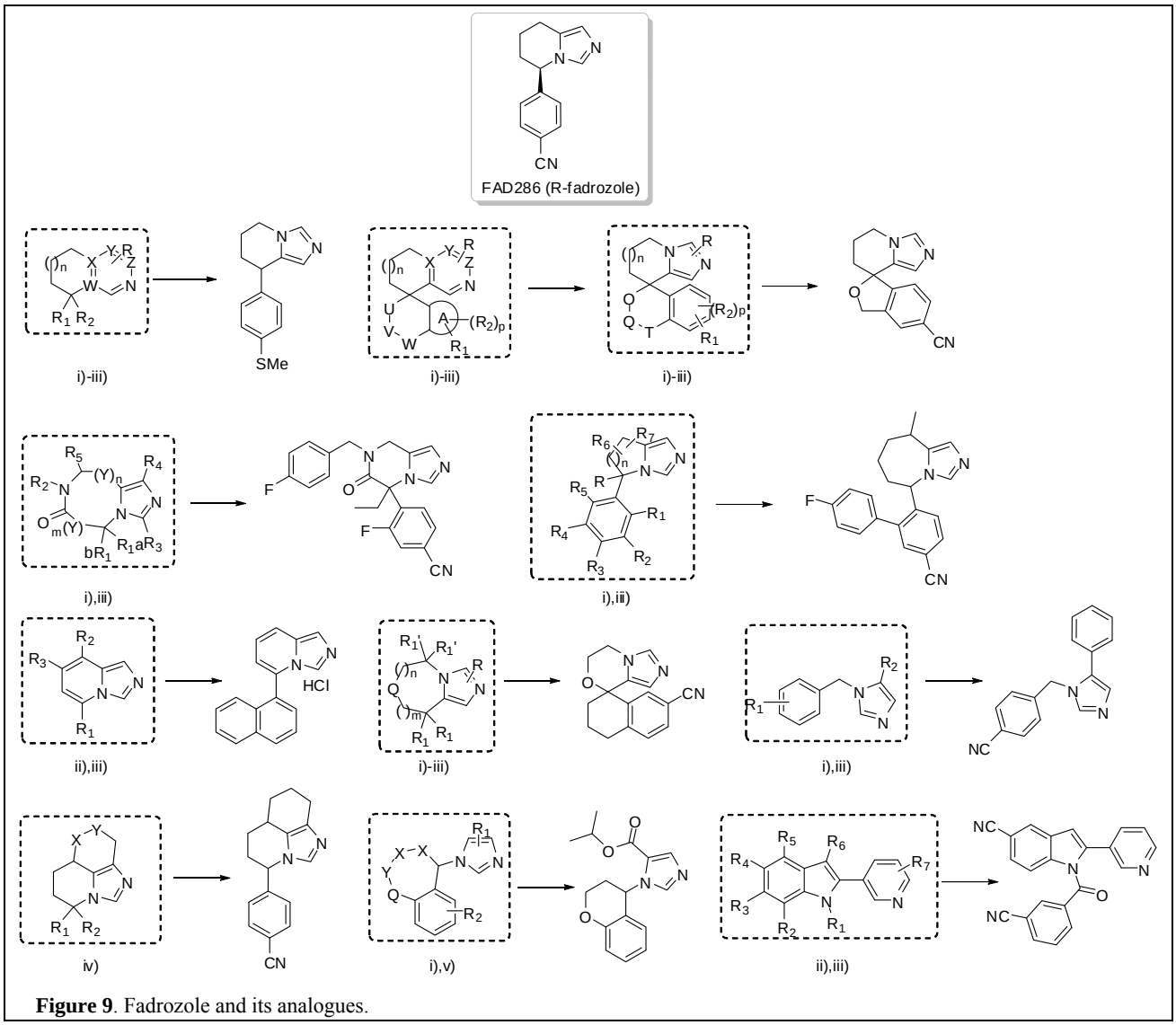


Figure 9. Fadrozole and its analogues.

1.2.4.a Fadrozole and Its Analogues

Fadrozole has been developed as an aromatase (CYP19) inhibitor by Novartis for the treatment of postmenopausal breast cancer. Besides the potency toward CYP19, fadrozole shows potent inhibition of CYP11B2. Its two enantiomers show distinct potency toward CYP11B2. Among them, R-enantiomer prefers inhibiting CYP11B2 (IC_{50}: CYP11B2 = 6.0 nM, selectivity factor of $IC_{50\ CYP11B1}$ / $IC_{50\ CYP11B2}$ = 20), whereas S-enantiomer takes the preference for CYP11B1 (IC_{50}: CYP11B2 = 171 nM, selectivity factor of $IC_{50\ CYP11B1}$ / $IC_{50\ CYP11B2}$ = 0.23)[53]. In recent studies, fadrozole and its enantiomers were used as model compounds in in vivo tests to investigate their impact on aldosterone levels and thus to study their beneficial effects on the improvement of diseases related to aldosterone. Later on, modifications of the fadrozole backbone from different directions were made in order to find CYP11B2 inhibitors with a good selectivity profile by Novartis[54], Speedel[55], and others[56]. Generally, five optimization approaches were carried out. i) direct introduction of substituents to phenyl and / or imidazolyl and / or cyclohexyl moieties; ii) replacement of phenyl and / or imidazolyl by other cycle or N-containing heterocycle, respectively; iii) replacement of cyclohexyl by other substituted cycles or remove of it; iv) fusion of additional cycles (aliphatic, aromatic or hetero cycles) to imidazolyl; v) rearrangement of the three cycles. Usually, these approaches are employed in combination. Through these optimizations, many CYP11B2 inhibitors are obtained with strong potency and significantly improved selectivity.

1.2.4.b N-containing Heterocycle Substituted Methylene Tetrahydronaphthalenes or Dihydroindenes

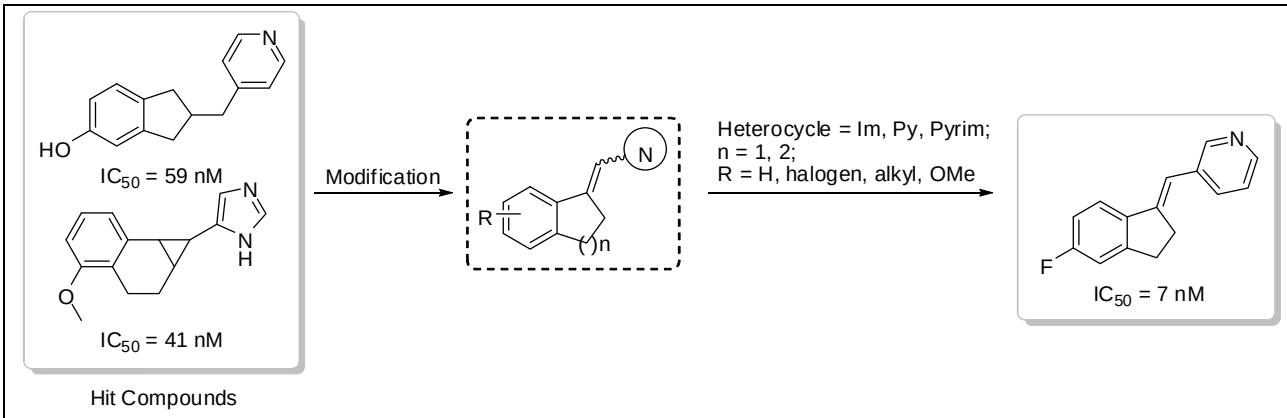


Figure 10. N-containing heterocycle substituted methylene tetrahydronaphthalenes or dihydroindenes

In our group, the hit compounds (Figure 10) were identified by screening an in-house CYP library containing approximately 500 compounds[57–62]. Through further modification of the hits, a series of N-containing heterocycle substituted methylene tetrahydronaphthalenes or dihydroindenes were identified as potent (IC_{50} < 10 nM) and selective (SF > 100 versus CYP11B1) CYP11B2 inhibitors. Moreover, *Z*-isomers were found to be more potent than the corresponding *E*-isomer[63,64].

1.2.4.c Pyridinyl Naphthalenes and Indenes: Semi-unsaturation and Heteroatom Insertion

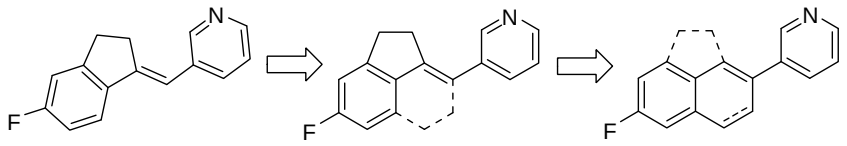


Figure 11. Design conception of pyridinyl naphthalenes.

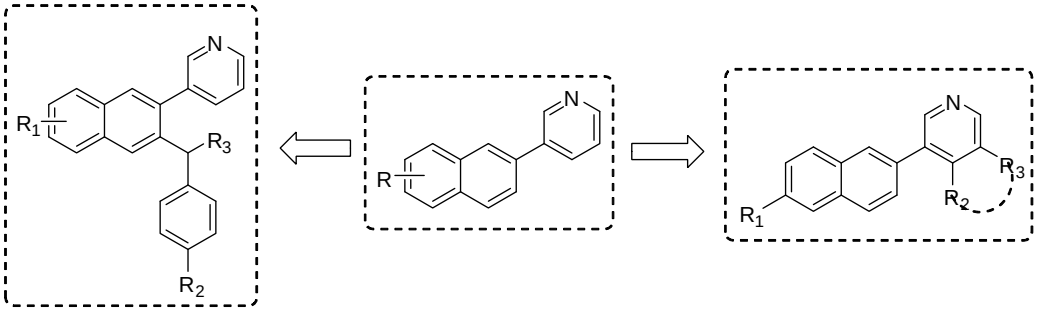


Figure 12. Optimization of pyridinyl naphthalene: benzyl substitution on naphthalene core, and substituents on pyridine.

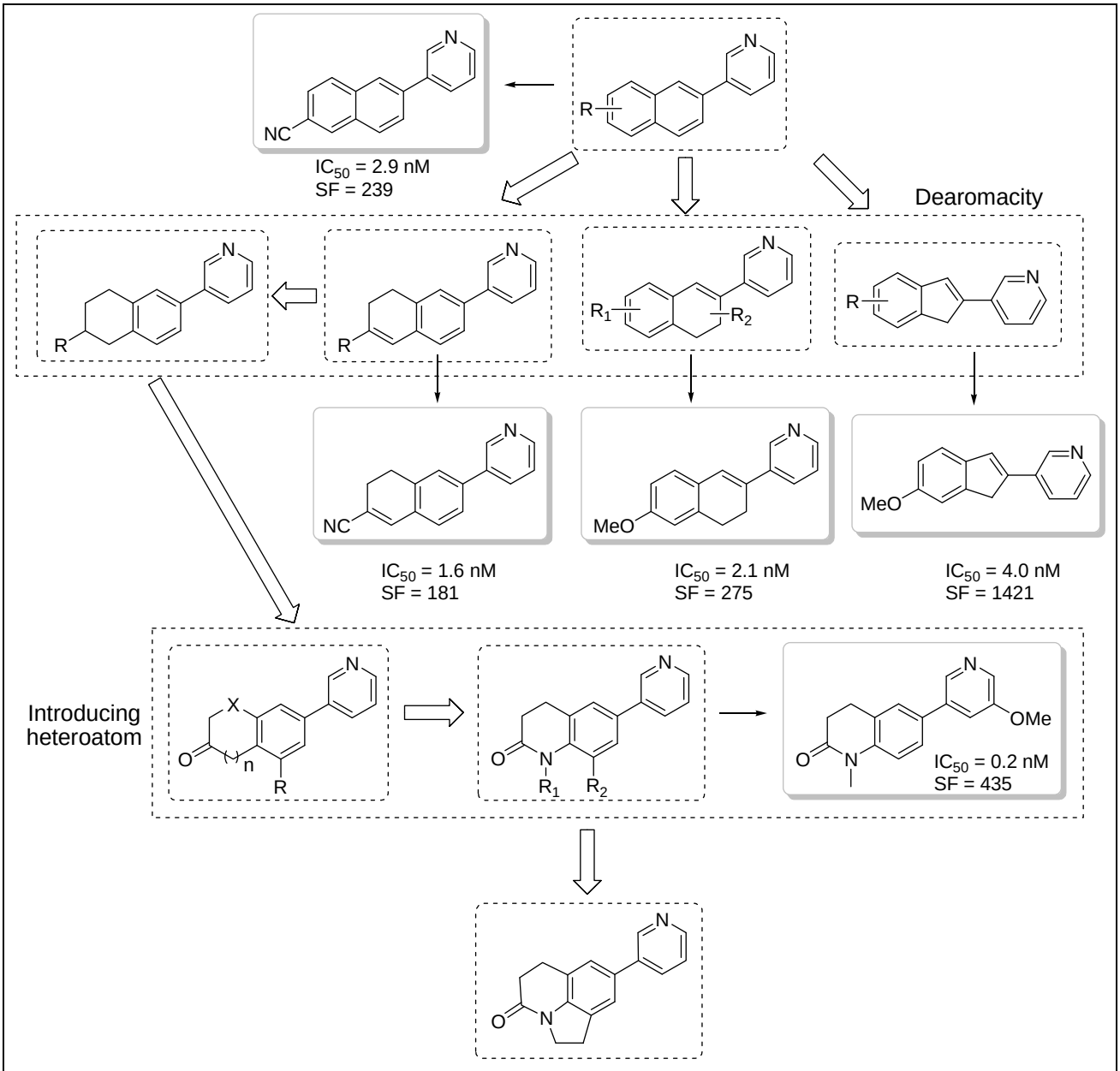


Figure 13. Optimization of pyridinyl naphthalene and indene: semi-unsaturation and heteroatom inserting.

Further structural modification in our group was made by annulation of the exocyclic double bond in 3-pyridinyl methylene dihydroindenes into a new cycle fused to the phenyl ring and thereafter removal of the original five-membered ring, thus affording pyridinyl naphthalenes (Figure 11) [65,66]. The resulting type of compounds were very potent and selective, however, they exhibited strong inhibition against hepatic CYP1A2. This means these compounds are not feasible to be developed to drug candidates due to the expected drug-drug interactions. Direct introduction of additional substituents to the pyridinyl naphthalene core includes two approaches: benzyl substitution[67] on the naphthalene core, and substituents on the pyridine[68] (Figure 12). These modifications made beneficial effects on both potency and selectivity profile toward other steroidogenic and hepatic enzymes. In addition, on the basis of the knowledge that CYP1A2 predominantly hydroxylates those substrates with planar structure, it seemed to be reasonable to decrease CYP1A2 inhibition by dearomatisation of the naphthalene core and thus reducing the planar structure of the molecule. Several attempts were made for this purpose. The naphthalene core was semi-saturated to a certain degree either at the left ring or the right part (Figure 13) [66,68,69]. Moreover, introduction of heteroatoms into the saturated ring[69] and / or fusion of a third ring to the tetrahydronaphthalene[70] leads to distinct structures with increased solubility and improved pharmacokinetic properties. Besides, a ring contraction of naphthalene resulting in indenes (Figure 13)[66], led to the most selective CYP11B2 inhibitor toward CYP11B1. After these optimizations, not only the inhibitory potency of CYP1A2 was significantly reduced, but also the potency and selectivity were improved.

1.3 CYP11B1 Inhibitors and Cushing's Syndrome

1.3.1 Cortisol: physiology and pathology

Cortisol is a major human glucocorticoid. Apart from primarily playing an important role in the regulation of glucose metabolism, cortisol is also pivotal in development, metabolism, neurobiology, cell growth and apoptosis, as well as other biological functions[71]. In genomic manner, cortisol action is mediated by glucocorticoid receptor (GR), a member of nuclear receptor superfamily of ligand-dependent transcription factors. GR is expressed in almost all cells, and is located in the cytoplasm associated with a multiprotein chaperone complex, of which the key component is the heat shock protein Hsp90, in its inactive state. After cortisol binding to the glucocorticoid receptor, GR is activated, and renders hyper-phosphorylated[72]. The activated GR dissociates from the multiprotein complex, and leads to the translocation of ligand-binding GR from cytoplasm to nucleus. Afterwards, GR binds to glucocorticoid-responsive elements (GREs), and then activates the target gene transcription and thus unfolds physiological effects. In addition, some gene transcriptions require not only binding to GREs but also association with glucocorticoid responsive units[73]. Besides, the GR complex can also repress the gene transcription by interaction between the GR complex and negative GREs[74]. Expect for genomic action, rapid nongenomic mechanisms of cortisol action on prolactin were also observed through interactions with the plasma membrane[75].

However, chronic hypersecretion of circulating cortisol by adrenal cortex causes Cushing's syndrome (also termed as hypercortisolism)[76]. According to the causes of high cortisol levels, Cushing's syndrome can be categorized to ACTH-dependent and ACTH-independent Cushing's syndrome[77]. ACTH-dependent

Cushing's syndrome is characterized by chronic hypersecretion of ACTH, which consequently stimulates growth of adrenal glands and production of cortisol. According to different locations resulting in hypersecretion of ACTH, ACTH-dependent Cushing's syndrome can be separated into Cushing's disease, which stems from pituitary adenoma, and ectopic Cushing's syndrome, which can be seen in diseases like small-cell lung cancer, pancreatic islet cell tumor, pheochromocytoma, thymoma and medullary thyroid cancer. ACTH-independent Cushing's syndrome is characterized by excess production of cortisol, most commonly resulting from adrenal tumors. Symptoms of Cushing's syndrome include central obesity, hyperhidrosis, increased thirst, thinning of the skin, fatigue, backache, headache, muscle atrophy, hypertension, impotence, infertility, osteoporosis, gastrointestinal disturbances, opportunistic infections and impaired wound healing, hyperglycemia, and neuropsychological disturbances.[76]

1.3.2 Regulation of Cortisol Secretion

Cortisol is biosynthesized from 11-deoxycortisol in the final step catalyzed by 11ß-hydroxylase (CYP11B1), which is located in the zona fasciculata / reticularis of adrenal cortex[78]. Normally, the production and secretion of cortisol is circadian, and controlled acutely by ACTH via a negative feedback loop of hypothalamic-pituitary-adrenal axis. The hypothalamus, as a sensor, varies as the environmental stimuli. Two hypothalamic neurohormones, corticotrophin-releasing hormone (CRH) and arginine vasopressin (AVP), are transported to the anterior pituitary gland, and interact with their specific receptors to stimulate the release of the ACTH to blood circulation. Subsequently, ACTH interacts with melanocortin receptor type II in the adrenal cortex to thereby result in the production and secretion of cortisol. In turn, the elevated levels of cortisol function as a suppressive factor of the release of CRH and AVP. Additionally, increase in potassium was reported to have a positive effect on the secretion of ACTH and cortisol[79].

1.3.3 Treatment of Cushing's Syndrome — State of Art

Among the different subtypes of Cushing's syndrome, Cushing's disease accounts for ca. 70% of patients, ectopic Cushing's syndrome for ca. 10%, and ACTH-independent Cushing's syndrome for ca. 20% [80]. The mortality and morbility of Cushing's syndrome drive the attention to approaches to its effective treatment. Although surgery is the primary therapy for the treatment of patients with Cushing's syndrome, not every patient is endurable or willing to undergo a surgical therapy and thus the convenient medical therapy becomes important. Furthermore, severe complications of the syndrome, resulting in increased risk of surgery, as well as recurrent cases after operation can be largely reversed by medical therapy. The medical therapy can be employed by modulation of ACTH release at pituitary gland or ectopic site, blockage of the glucocorticoid receptors and inhibition of cortisol production at adrenal cortex.

1.3.3.a ACTH Release Neuromodulators

The medical agents in clinic impact on the release of CRH and thus ACTH secretion and include cyproheptidine, bromocriptine, valproic acid and octreotide (Figure 14). Cyproheptidine is an unselective 5-hydroxytryptamine receptor blocker, and also exhibits inhibition of histamine, dopamine and cholinergic activity. The efficacy of normalization of cortisol levels by regulation of ACTH secretion was evaluated[81,82].

Bromocriptine, as a dopamine agonist, is not only used for the treatment of Cushing's disease, but also approved for the treatment of Parkinson's disease, hyperprolactinaemia, neuroleptic malignant syndrome, and type 2 diabetes. In order to reach responsive effect in the patients with Cushing's disease, a high daily dose of more than 35 mg has been suggested[83]. However, another study indicated that bromocriptine was similarly effective to placebo in the rapid decrease in cortisol levels in patients with Cushing's disease[84]. Sodium valproate, a gamma aminobutyric acid (GABA)-transaminase inhibitor, can increase the GABA levels, which may induce the decrease in the CRH secretion or direct effect on pituitary tumor[85]. The mechanism in which octreotide, a somatostatin analogue, effects on the reduction of ACTH release has not been well demonstrated. The suppressive effects of octreotide on ACTH secretion are controversial[86–90]. Moreover, octreotide need several times of subcutaneous injections per day due to its short half time, and thus its application is limited in clinic. In addition, severe side effects are not avoided, due to the fact that these neuromodulator also play import roles in multiple targets.

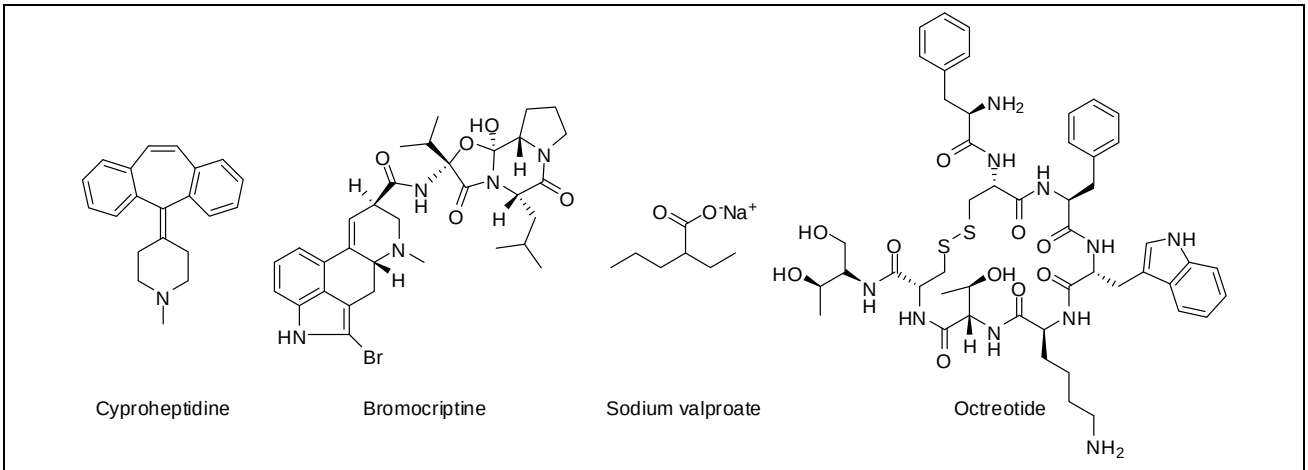


Figure 14. Typical ACTH release neuromodulators: 5-hydroxytryptamine receptor blocker cyproheptidine, dopamine D2 receptor agonist bromocriptine, GABA-ergic compound sodium valproate and somatostatin analogue octreotide.

1.3.3.b Glucocorticoid Receptor Antagonist

Through blockage of GR-mediated effects, GR antagonists can make some benefits on improvement of metabolic or psychological disorders related to hypersecretion of cortisol. Mifepristone (Figure 15) is an unselective glucocorticoid receptor antagonist, and competitively antagonizes both GR and progesterone receptor (PgR). Due to the antiprogestagenic activity, mifepristone is also implemented as an abortifacient agent in clinic. Afterwards, several classes of mifepristone derivatives have been reported in order to elevate the selectivity toward GR[91], comprising 11-monoaryl derivatives, 11,21-bisaryl derivatives and 11-aryl,16-hydroxy derivatives (Figure 15). Although the selectivity toward GR was improved through deterioration of

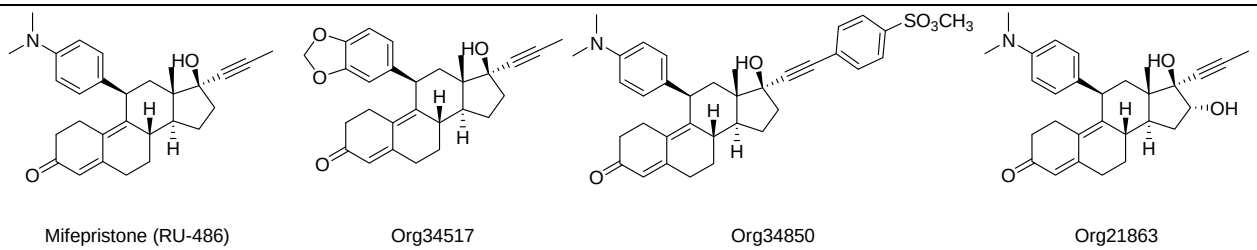


Figure 11. Glucocorticoid receptor antagonist mifepristone and its derivatives.

the affinity to GR, these derivatives showed less potency in vivo in comparison to mifepristone.

1.3.3.c Steroidogenesis Inhibitors

Steroidogenesis inhibitors can directly decrease overproduction of cortisol. Generally, steroidogenesis inhibitors consist of single enzyme inhibitors and multiple enzyme inhibitors. Metyrapone and trilostane are regarded as primarily inhibiting one ezymatic reaction by inhibition of CYP11B1 and 3ß-hydroxysteroid dehydrogenase (3ß-HSD) respectively, whereas, etomidate (CYP11B1, CYP11B2), ketoconazole (CYP17, CYP11A1, CYP11B1, CYP11B2), aminoglutethimide (CYP11A1, CYP17, CYP21B, CYP11B1, CYP11B2 and CYP19), and mitotane (CYP11A1, CYP11B1, CYP11B2, 3ß-HSD) inhibit several enzymatic steps[92].

By blockage of the final step of cortisol synthesis by inhibition of the responsible enzyme CYP11B1, metyrapone can rapidly reduce of cortisol levels. However, the selectivity toward its highly homologous enzyme CYP11B2 needs to be improved. A recent study in our group uncovered a series of CYP11B2 inhibitors by modification of metyrapone, which exhibited improved potency and selectivity toward CYP11B2[93].

Trilostane is an inhibitor of 3ß-HSD, involved in the conversion of pregnenolone or 17α-hydroxy-pregnenolone to progesterone or 17α-hydroxyprogesterone. Since trilostane blocks the early step of the steroidogenetic reactions, it impacts on the production of important hormones, including glucocorticoids, mineralocorticoids, estrogens and androgens. Similarly, multiple enzyme inhibitors effect on several steps of steroidogenesis due to poor selectivity, and thus result in severe side effects. Etomidate is an anesthetic agent, which is also the only drug used for the treatment of Cushing's syndrome and is administrated intravenously. Recently, a series of etomidate derived compounds was reported with potent inhibition of CYP11B1[94]. Other multiple enzyme inhibitors, such as ketoconazole and aminoglutethimide, were initially used as an antifungal agent, or an anticonvulsant, respectively. Mitotane, an analogue of an insecticide, can not only inhibit steroidogensis, but was also found to lead to adrenocortical atrophy and necrosis and thus was used for the treatment of adrenocortical carcinoma[92].

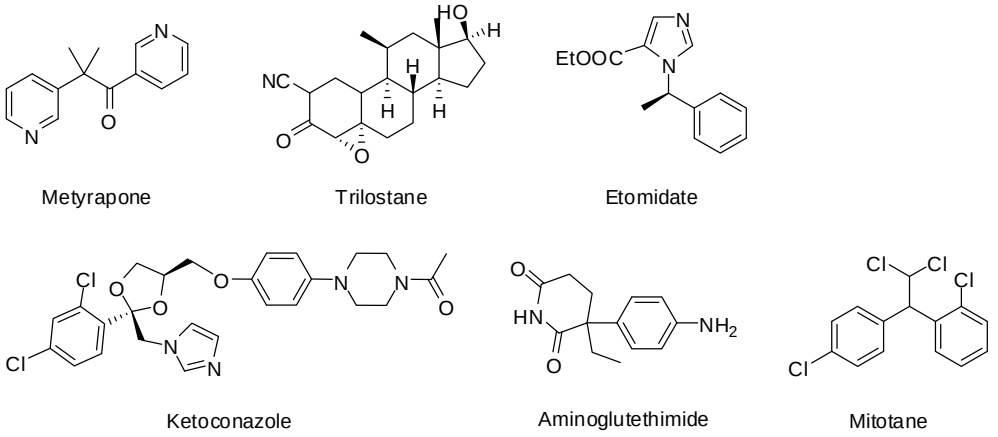


Figure 14. Steroidogenesis Inhibitors: metyrapone and trilostane, etomidate, ketoconazole, aminoglutethimide, and mitotane.

1.4 Dual Inhibitor CYP19 / CYP11B2 to Cure Cardiovascular Diseases in Breast Cancer Patients

With respect to the pharmaceutical approach of selective multi-target-directed ligands used for the treatment of complex diseases, it has lower risk of drug-drug interaction and better compliance compared to the therapy of drug cocktails and multicomponent drugs. This drug discovery paradigm, especially dual inhibitors or dual antagonists, has already been employed for the treatment of different diseases, e.g. nonsteroidal antiinflammatory drugs inhibiting both cyclooxygenase-2 and 5-lipoxygenase, dual inhibitors of angiotensin converting enzyme (ACE) and neutral endopeptidase (NEP) used for the treatment of hypertension and CHF as well as PPARα/PPARγ dual agonists for metabolic diseases[95].

Worldwide, breast cancer (BC) accounts for ca. 10% of all cancer incidences in female, rendering it the most common type of non-skin cancer among women. Due to earlier diagnosis by BC screening[96] and especially the implementation of adjuvant endocrine therapy in the earlier stage[97], the mortality of BC has been markedly reduced. Endocrine treatment of BC stemmed from the fact that estrogen regulates the proliferation of hormone sensitive BC[97], which comprise over 60% of all BC cases. Therefore, blockage of estrogen action has been regarded as a promising approach for the treatment of hormone sensitive BC. Selective estrogen receptor modulators (SERM) [98], e.g. tamoxifen and raloxifen (Figure 15), were introduced into the clinic for the treatment of hormone-positive early BC in postmenopausal women. However, the poor risk / benefit profiles lead to discontinuous application of tamoxifen for over five years. In addition, severe side effects such as endometrial cancer and thrombosis were reported[99]. On the other hand, aromatase inhibitors have been proposed on the basis that aromatase (CYP19) is the pivotal enzyme catalyzing the final step of estrogen biosynthesis from testosterone or androstenedione. Thus, inhibition of CYP19 can directly decrease estrogene production, and consequently stops tumor proliferation. The third generation aromatase inhibitors, e.g. anastrozole, letrozole and exemestane (Figure 15), were launched to the market, and exhibited better efficacy and tolerability than tamoxifen. Aromatase inhibitors are today first-line therapeutics for

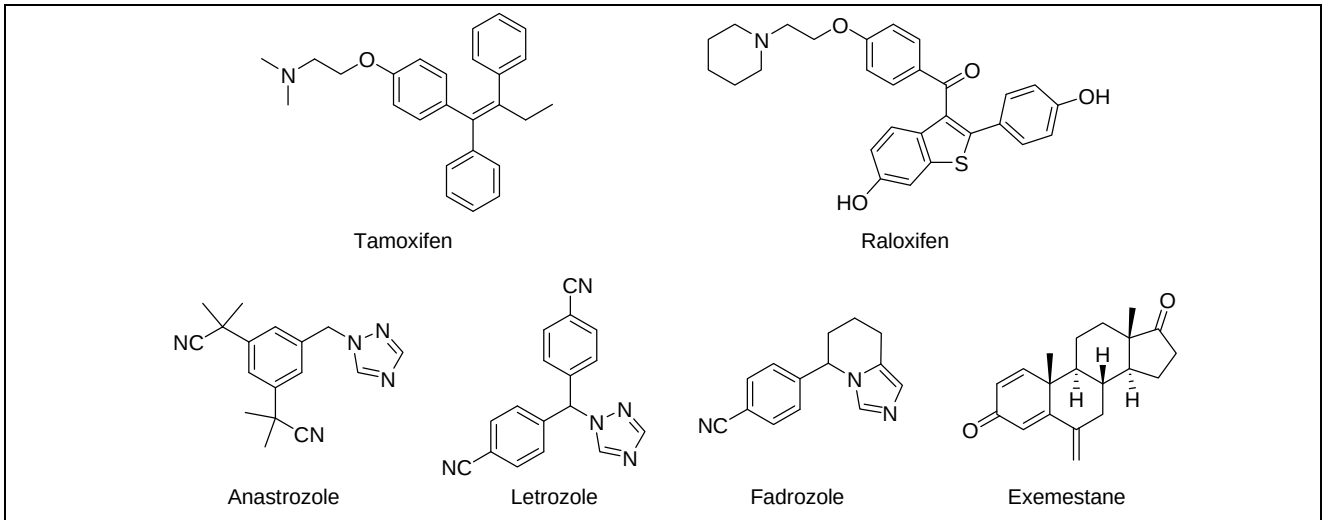


Figure 15. Selective estrogen receptor modulators: tamoxifen and raloxifen; and aromatase inhibitors: anastrozole, letrozole, fadrozole and exemestane.

BC in postmenopausal women. It is reported that the plasma estrogen concentration is significantly reduced to undetectable levels after administration of aromatase inhibitors[100,101]. Moreover, clinic trials elucidated that aromatase inhibitors as adjuvant therapy dramatically ameliorate disease-free survival and relapse-free survival[102–105].

However, a study indicates that non-BC-related deaths were more common than BC-specific deaths in those five-year breast cancer survivors, especially in older patients[106]. It also demonstrates that cardiovascular diseases (CVD) among other diseases were found as a major cause of non-BC-related deaths[106]. Therefore, it is of importance to deal with CVD in order to improve the overall survival of BC patients. Another observation that estrogen deficiency is closely associated with CVD stems from the fact that CVD incidences in post-menopausal women are 3-fold higher than those in pre-menopausal women at the same age[107,108]. Recent studies have shown that estrogens show beneficial effects on the heart[109–115] and kidney[116]. Estrogen treatment prevents the development of post-myocardial infarction remodelling[113] and improves left ventricular hypertrophy and structural and functional remodelling[114,115].

Obviously, the treatment of post-menopausal BC patients by aromatase inhibitors can further decrease the estrogen production to very low levels, and consequently gives rise to even higher risk of CVD. It has been described above that high concentrations of aldosterone lead to the progression of severe CVD. Estrogen depletion has been proved to not only directly increase circulating aldosterone levels, but to upregulate other RAAS components, such as renin, Ang II, ACE and AT_1 receptor, which further stimulate aldosterone secretion[117–122]. Moreover, estrogen deficiency also increases the concentration of potassium in the plasma which is a key factor resulting in the promotion of aldosterone secretion[123,124]. Therefore, abnormally high levels of aldosterone as a consequence of estrogen deficiency exhibit detrimental effects on the progression of CVD.

It is rational to propose that normalizing of aldosterone levels in BC patients under treatment of aromatase inhibitors should be a promising strategy to manage the accompanying CVD[125]. As CYP11B2, a member of the same CYP superfamily as CYP19, is the key enzyme in aldosterone biosynthesis catalyzing the terminal step, dual inhibition of CYP11B2 and CYP19 should be superior to reduce aldosterone levels and thus to improve the overall survival of BC patients.

2 Aim and Scope of the Work

Since steroidogenic CYP enzymes are closely associated with severe diseases, they are considered as promising targets. As discussed in the introduction, inhibition of CYP11B2 can be used for the treatment of cardiovascular diseases caused by abnormally high aldosterone concentrations, while inhibition of CYP19 is an important treatment for breast cancer, and CYP11B1 inhibition is regarded as a good pharmaceutical therapy for patients with Cushing's syndrome.

2.1 CYP11B2 Inhibitors

Currently, two main therapies are clinically employed to suppress the components of RAAS, including ACE inhibitors and MR antagonists for the treatment of patients with hypertension, and / or congestive heart failure. In order to overcome the drawbacks of these two therapies, such as "aldosterone escape" by ACE inhibitors and hyperkalemia by MR antagonists, respectively, selective CYP11B2 inhibitors are proposed to be a new approach. Due to the homology among members of CYP enzymes, selectivity is regarded as a challenge in order to avoid side effects caused by interaction with other steroidogenic CYP enzymes like CYP11B1, CYP17 and CYP19 as well as important hepatic enzymes such as CYP1A2. In section 3.I and 3.II, novel potent and selective CYP11B2 inhibitors have to be designed, synthesized and evaluated.

2.2 CYP19 / 11B2 Dual Inhibitors

The application of aromatase inhibitors to post-menopausal breast cancer patients increases the risk of cardiovascular diseases, which is believed to be caused by the estrogen deficiency and mediated to a large extent through abnormally high concentration of aldosterone. Dual inhibitors of CYP19 and CYP11B2 are therefore proposed as a novel therapy for an adjuvant therapy to reduce the CVD risk for these patients. Although the homology among CYP superfamily often leads to a challenge of selectivity, it renders the possibility of dual inhibition. In section 3.III, potent and selective dual CYP19 / CYP11B2 inhibitors are developed.

2.3 CYP11B1 Inhibitors

Apart from surgical therapy to remove some adrenal or pituitary tumors, CYP11B1 inhibition as a pharmaceutical approach to block cortisol biosynthesis is an important alternative in the treatment of Cushing's syndrome. Although several unselective CYP11B1 inhibitors are employed clinically, they are accompanied by severe side effects. This situation of absence of effective and safe therapy for the treatment of Cushing's syndrome encourages us to look for more potent and more selective CYP11B1 inhibitors. In section 3.IV, novel imidazol-1-ylmethyl substituted 1,2,5,6-tetrahydro-pyrrolo[3,2,1-ij]quinolin-4-ones as potent and selective CYP11B1 inhibitors are designed, synthesized and tested.

3 Results and Discussions

The design, syntheses and bio-evaluation of CYP11B2 inhibitors, dual inhibitors CYP19 / 11B2 as well as CYP11B1 are described in detail in the following:

3.I. 3-Pyridinyl Substituted Aliphatic Cycles as CYP11B2 Inhibitors: Increased Selectivity over CYP1A2 by Dearomatisation of the Core

Introduction

Cardiovascular diseases are the leading cause of death in the United States and the majority of the European countries. Some forms of hypertension, congestive heart failure (CHF) and myocardial fibrosis (MF) are closely associated with high aldosterone levels.[1–3] Phisiological aldosterone concentrations are crucial for the regulation of electrolyte and volume homeostasis. After binding to mineralocorticoid receptors (MR), aldosterone promotes the retention of sodium and water at the expense of potassium excretion, subsequently resulting in the increase of blood volume and hypertension. Moreover, high aldosterone levels also stimulate synthesis and accumulation of collagens in cardiac fibroblasts leading to MF. The resulting increase in myocardial stiffness causes diastolic dysfunction and ultimately heart failure.[3]

Therefore, deprivation of aldosterone from its pathological effects is a feasible therapeutic approach to treat the related diseases. Currently, two main pharmacotherapies are clinically implemented to suppress the components of the renin-angiotesin-aldosterone system (RAAS), which controls the secretion of aldosterone via a negative feedback loop, including angiotensin-converting-enzyme (ACE) inhibitors such as enalapril (Figure 1) and MR antagonists like spironolactone and eplerenone (Figure 1). ACE inhibitors are used for the treatment of hypertension and CHF by down-regulation of angiotensin II and subsequent aldosterone secretion. However, long-term suppressive effects of ACE inhibitors on plasma aldosterone levels are weakened due to the phenomenon known as “aldosterone escape”.[5, 6] Although clinical studies revealed that blockade of MR by spironolactone has reduced the risk of both morbidity and mortality in patients with severe heart failure, the MR antagonists show severe adverse effects such as gynaecomastia or breast pain due to their steroidal structure exhibiting a residual affinity to other steroid receptors.[7] Despite the fact that eplerenone as a selective MR antagonist achieves some improvement in terms of side effects as compared to spironolatone, severe hyperkalemia and less potency have been reported.[8] Furthermore, treatment with MR antagonists leaves high levels of aldosterone unaffected,[9] which can result in further exacerbation of heart function in a MR independent nongenomic manner.[10] CYP11B2 is a mitochondrial cytochrome P450 enzyme catalyzing the conversion of 11-deoxycorticosterone to aldosterone in three consecutive steps (Scheme 1).[11] Its inhibition was proposed as a new strategy for the treatment of aldosterone related cardiovascular diseases as early as 1994.[12] Recent *in vivo* studies in rats have demonstrated that CYP11B2 inhibitors can reduce plasma aldosterone levels.[13, 14] Long-term administration of FAD286 (R-enantiomer of

fadrozole, Figure 1) to rats with heart failure improves cardiac haemodynamics and cardiac function, which is more significant than those by spironoloactone.[15]. However, FAD286 also shows strong inhibition of CYP11B1 and CYP19, thus urging us to design selective CYP11B2 inhibitors.

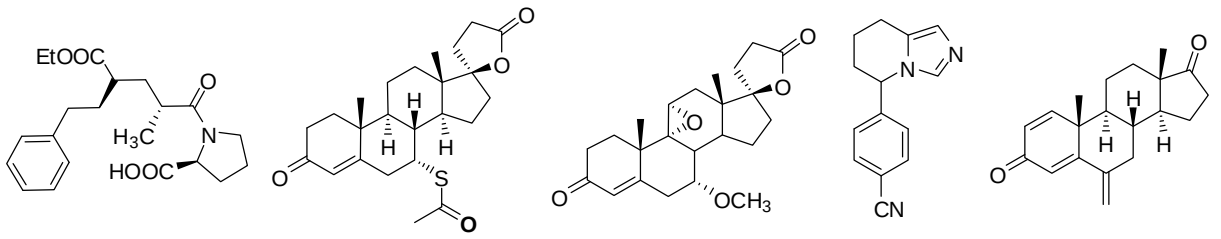
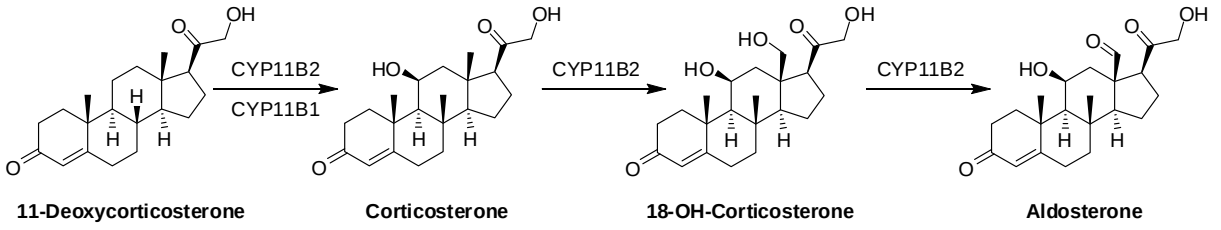


Figure 1. Structures of ACE inhibitor Enalapril, MR antagonists Spironolactone and Eplerenone, CYP11B2 inhibitor Fadrozole and aromatase inhibitor Exemestane.

Our group has designed and synthesized several series of CYP11B2 inhibitors.[16] These compounds not only exhibited potent inhibition toward CYP11B2, but also showed good selectivity over CYP11B1, which is the key enzyme involved in the glucocorticoid biosynthesis. This selectivity is very difficult to achieve due to the high homology up to 93% between these enzymes. However, some of these potent compounds showed strong inhibition of CYP1A2, which is probably due to the planar aromatic structure of the molecules. Therefore, in this study the Dearomatisation of the core was performed to improve the CYP1A2 selectivity leading to a series of 3-pyridinyl substituted aliphatic cycles **1** – **21**. The percent inhibition and IC_{50} values of the inhibitors for CYP11B2 and CYP11B1 are presented in comparison to fadrozole. Inhibition of CYP1A2 was tested for potent and selective compounds **2**, **4**, **7**, **8** and **10**.



Scheme 1. Biological synthesis of aldosterone catalyzed by CYP11B2.

Inhibitor Design Concept

In the last few years, a wide range of compounds were designed as CYP11B2 inhibitors[16] based on the mechanism that an inhibitor sp^2 hybrid N coordinates to the heme iron located in the protoporphyrin center of the enzyme. This mechanism was first identified for aromatase inhibitors,[17] but subsequently was proven to be valid for inhibitors of other steroidogenic enzymes such as CYP17.[18] Since all cytochrome P450 (CYP) enzymes, not only steroidogenic but also hepatic CYPs, consist of a heme as the catalyzing unit, they are potential targets of inhibitors acting through this mechanism. Therefore, it is crucial to develop CYP11B2 inhibitors exhibiting selectivity toward these enzymes, especially CYP11B1 which shows a homology of approximately 95% to the target enzyme. Another focus of selectivity is hepatic CYP enzymes due to their important roles in the metabolism of drugs and xenobiotics. Our previously designed inhibitors are quite potent toward CYP11B2 and very selective over CYP11B1, however, their selectivity toward CYP1A2 still

needs to be improved. CYP1A2, which accounts for approximately 13% of totally expressed CYP450 enzymes in the liver, is responsible for metabolizing neutral or basic planar substances.[19] Since the heterocycles providing the sp^2 hybrid N must not be modified, the key to selectivity lies in the hydrophobic core. After we had seen that partial dearomatisation of naphthalene compound **I**[16e] (Figure 2), showing a strong CYP1A2 inhibition of 98% at a concentration of 2 µM (Table 3), resulted in a decrease in CYP1A2 inhibition (compounds **II**[16a] and **III**[16c], 80 and 73%, respectively), but did not change high inhibitory potency toward CYP11B2 and good selectivity against CYP11B1, we wanted in more detail to investigate these relationships. In the following we describe the influence of structural modifications of the core structure regarding aromaticity, planarity, structural flexibility/rigidity, cycle size, presence of H-bond forming groups and removal of bridge bonds on activity (CYP11B2) and selectivity (CYP11B1 and CYP1A2).

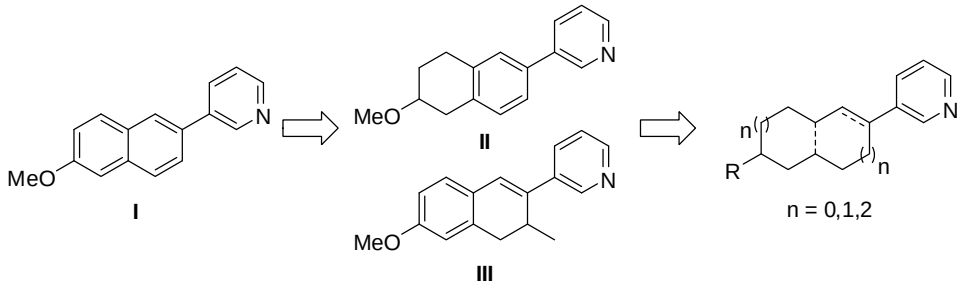


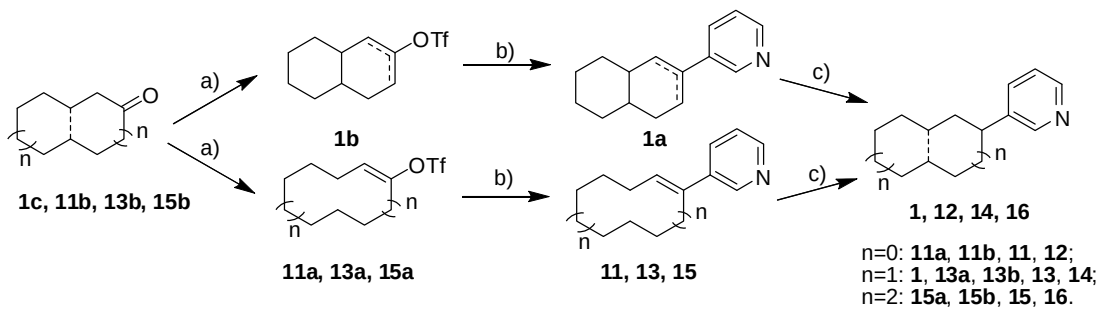
Figure 2. Inhibitors design.

Results and Discussion

Chemistry

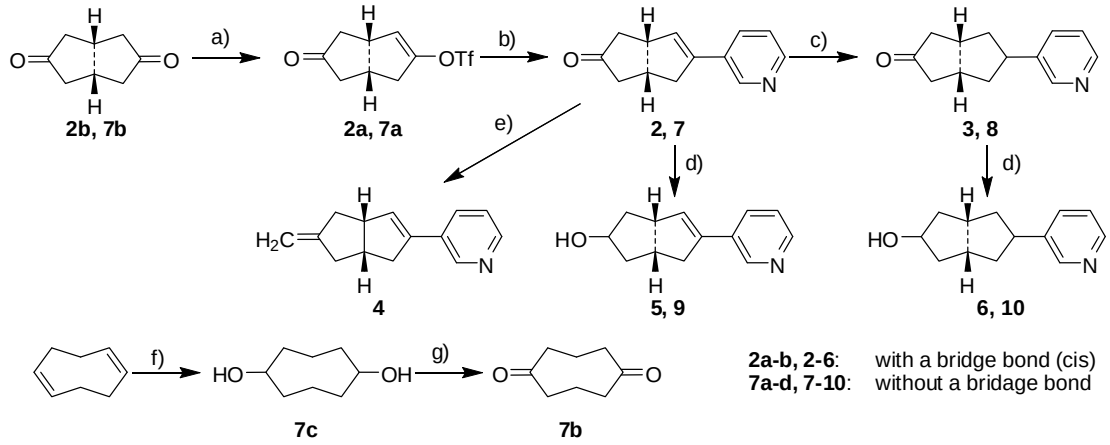
The synthetic strategy employed is shown in Scheme 2–5. The triflation[20] of the starting ketone compounds using trifluoromethanesulfonic anhydride and 2,6-di-*tert*-butyl-4-metyhylpyridine gave corresponding enol triflates. These were then coupled with 3-pyridyl boronic acid leading to the corresponding products with an α-double bond (**2**, **7**, **11**, **13**, **15**, **18** and **20**). The subsequent hydrogenation catalyzed by Pd / C was used to reduce the α-double bond and thereby to yield the saturated compounds (**1**, **3**, **8**, **12**, **14**, **16**, **19** and **21**). Several interesting points need to be addressed: **1b** obtained from the triflation of octahydronaphthalen-2(1*H*)-one was a mixture of two isomers with Δ 1, 2- or Δ 2, 3- double bond, 1 equivalent of trifluoromethanesulfonic anhydride needed to be strictly controlled in the triflation of diones **2b** and **7b**, which were converted to the respective ketones **2**, **3**, **7** and **8** following the general strategy described above. The latter were then reduced to the hydroxy compounds **5**, **6**, **9** and **10** using $NaBH_4$. Compound **3** underwent Wittig reaction to provide compound **4.** As for asymmetric (±) 9-methyl-5(10)-octaline-1,6-dione, the triflation occured selectively in 6-position with the rearrangement of the conjugated double bonds[21] resulting in **17a**, which after Suzuki coupling led to **17**. Compound **21** was obtained as an endo / exo mixture in a ratio of 5:1, which was tested without further separation.

Scheme 2 [a]



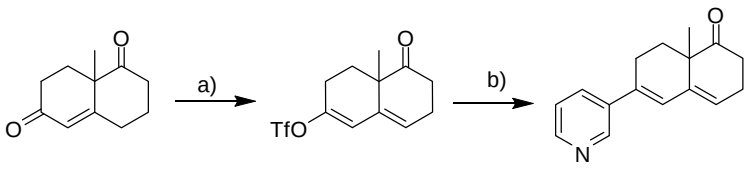
[a] Reagents and conditions: a) **Method A:** Tf_2O, CH_2Cl_2, 2,6-Di-*tert*-butyl-4-methylpyridine, 2 h; b) **Method B:** $Pd(PPh_3)_4$, Pyridine-3-boronic acid, Na_2CO_3, DME, H_2O, 90 °C, 2 h; c) **Method C:** 5% Pd / C, MeOH, H_2, RT, 2 d.

Scheme 3 [a]



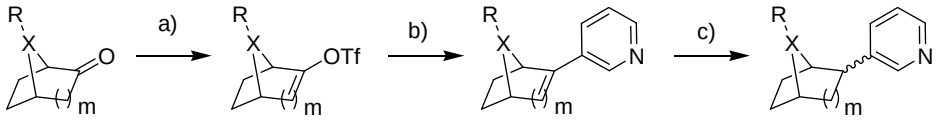
[a] Reagents and conditions: a) **Method A:** Tf_2O, CH_2Cl_2, 2,6-Di-*tert*-butyl-4-methylpyridine, 2 h; b) **Method B:** $Pd(PPh_3)_4$, Pyridine-3-boronic acid, Na_2CO_3, DME, H_2O, 90 °C, 2 h; c) **Method C:** 5% Pd / C, MeOH, H_2, RT, 2 d. d) **Method D:** $NaBH_4$, MeOH, RT, 2 h; e) CH_3PPh_3Br, n-BuLi, THF, 30 min at -78 °C, 2 h at RT, f) BH_3-THF, 3M NaOH, 35% H_2O_2, THF, g) PCC, CH_2Cl_2, reflux 2 d.

Scheme 4 [a]



[a] Reagents and conditions: a) **Method A:** Tf_2O, CH_2Cl_2, 2,6-Di-*tert*-butyl-4-methylpyridine, 2 h; b) **Method B:** $Pd(PPh_3)_4$, Pyridine-3-boronic acid, Na_2CO_3, DME, H_2O, 90 °C, 2 h.

Scheme 5 [a]



[a] Reagents and conditions: a) **Method A:** Tf_2O, CH_2Cl_2, 2,6-Di-tert-butyl-4-methylpyridine, 2 h; b) **Method B:** $Pd(PPh_3)_4$, Pyridine-3-boronic acid, Na_2CO_3, DME, H_2O, 90 °C, 2 h; c) **Method C:** 5% Pd / C, MeOH, H_2, RT, 2 d.

CYP11B1 and CYP11B2 inhibition

The synthesized compounds were tested for their inhibitory potencies against CYP11B2 and CYP11B1 with V79 MZh cells expressing the respective enzymes.[22] The results are presented in Table 1 with Fadrozole as a reference, which showed strong inhibition of these two enzymes with IC_{50} values of 0.8 and 6.3 nM toward CYP11B2 and CYP11B1, respectively.

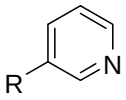
Intermediate **1a**, as a mixture of two isomers with the double bond at different positions, exhibited 96% inhibition of CYP11B2 at 500 nM and selectivity toward CYP11B1 with around 50% inhibition at the same concentration. However, these isomers could not be separated. Intermediate. After hydrogenation of compound **1a** to abondon isomery, the resulting saturated analogue **1**, however, showed decreased inhibitory potency of 76% against CYP11B2, but similar inhibition of CYP11B1 (55% at 500 nM). This result indicates the importance of the double bond to inhibitory potency and selectivity. To further investigate this phenomenon, ring size was reduced to avoid double bond isomery and a series of octahydropentalene analogues furnished with oxo, hydroxy or methylene was synthesized. Possessing an oxo group, the double bond analogue **2** exhibited an IC_{50} value of 34 nM and a selectivity factor of 8, whereas the corresponding saturated compound **3** was weaker (66%) and showed no selectivity. A similar result was observed for the hydroxy analogues **5** and **6**. The saturated hydroxy analogue **6** even showed preference for CYP11B1 (79%). Moreover, since exemestane with an exocyclic double bond can covalently bind to CYP enzymes to inhibit the enzyme in an irreversible manner[23] (Figure 1), which is an aromatase inhibitor in clinical use for the treatment of breast cancer, a methylene group was introduced into the molecule to increase the binding affinity. As expected, compound **4** turned out to be the most potent inhibitor in this study with an IC_{50} value of 6 nM and a selectivity factor of 34. The good selectivity over CYP11B1 indicates that this methylene rather specifically binds to amino acid residues present in CYP11B1.

Furthermore, to achieve more flexibility, the bridge bond was removed, resulting in a series of medium size cycles. When substituted by similar hydrogen bond forming groups such as oxo or hydroxy, the resulting compounds exhibited different activity profile patterns compared to the corresponding bridge compounds. For the ketone analogues **7** and **8**, the activities were slightly reduced, while the selectivities were maintained or even improved. Nevertheless, the hydroxy analogue with a double bond **9** showed an increased activity of 78% against CYP11B2, but the inhibition toward CYP11B1 also increased accordingly to 59%, leading to a deterioration of selectivity. On the contrary, the saturated compound **10** exhibited similar inhibitory potency (63%) of CYP11B2 but no selectivity. A similar structure activity relationship was observed for the compounds furnished with a hydrogen bond forming group (ketone or hydroxy). Regardless of the presence of the bridge bond, the analogues with double bond were always more potent and selective than the corresponding saturated analogues.

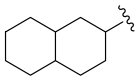
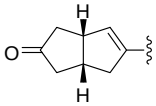
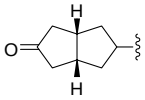
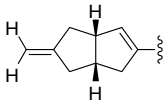
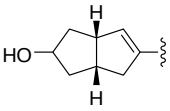
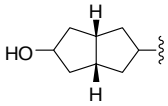
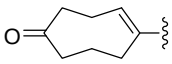
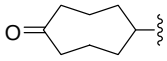
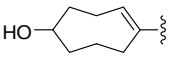
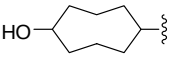
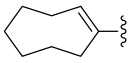
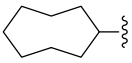
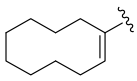
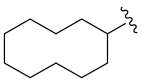
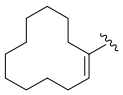
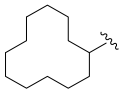
Moreover, the influence of the ring size on the inhibitory potency and selectivity was also investigated. It can be seen that the cyclodecane analogues, **13** and **14** showed inhibitory potency with IC_{50} values around 20 nM, regardless of the presence of the double bond. Nevertheless, the double bond analogue **13** was less selective compared to the saturated compound **14** (selectivity factor of 12 *vs.* 40 for compound **13** and **14**, respectively). After the ring size was reduced from cyclodecane to cyclooctane, the activity was slightly

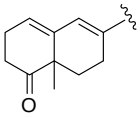
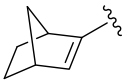
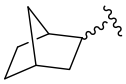
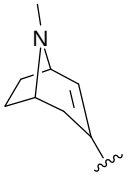
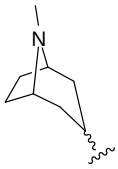
increased. The saturated compound **12** turned out to be very potent (IC_{50} = 21 nM), and this compound was

Table 1. Inhibition of human CYP11B1 and CYP11B2 by compounds 1–21.



1 - 21

Compd	R	% inhibition [a]		IC_{50} (nM) [b]		S. F. [e]
		CYP 11B2 [c]	CYP 11B1 [d]	CYP 11B2 [c]	CYP 11B1 [d]	
1		76	55	n.d.	n.d.	n.d.
2		79	46	34	282	8
3		66	62	n.d.	n.d.	n.d.
4		89	59	6	205	34
5		69	43	n.d.	n.d.	n.d.
6		69	79	n.d.	n.d.	n.d.
7		69	37	n.d.	n.d.	n.d.
8		53	27	n.d.	n.d.	n.d.
9		78	59	n.d.	n.d.	n.d.
10		63	67	n.d.	n.d.	n.d.
11		86	72	n.d.	n.d.	n.d.
12		90	30	21	1047	50
13		82	44	21	258	12
14		84	45	22	872	40
15		30	4	n.d.	n.d.	n.d.
16		44	22	n.d.	n.d.	n.d.

Compd	R	% inhibition [a] CYP 11B2 [c]	% inhibition [a] CYP 11B1 [d]	IC_{50} (nM) [b] CYP 11B2 [c]	IC_{50} (nM) [b] CYP 11B1 [d]	S. F. [e]
17		52	33	n.d.	n.d.	n.d.
18		36	19	n.d.	n.d.	n.d.
19		76	75	n.d.	n.d.	n.d.
20		17	10	n.d.	n.d.	n.d.
21		15	5	n.d.	n.d.	n.d.
Fadrozole				0.8	6.3	8.3

[a] Mean value of at least two experiments, standard deviation usually less than 10%; inhibitor concentration, 500 nM. [b] Mean value of at least four experiments, relative standard deviation usually less than 25%; n.d. = not determined. [c] Hamster fibroblasts expressing human CYP11B2; substrate deoxycorticosterone, 100 nM. [d] Hamster fibroblasts expressing human CYP11B1; substrate deoxycorticosterone, 100 nM. [e] IC_{50} CYP11B1/IC_{50} CYP11B2; n.d. = not determined.

also the most selective inhibitor throughout this study. The increase of the ring size to cyclododecane was not tolerated; the inhibitory activity of resulting compounds **15** and **16** was significantly reduced to less than 45%. Remarkably, the influence of the double bond in this case is totally contrary to that of compounds with hydrogen bond forming groups. These reverse effects are most likely a consequence of the different orientations of the compounds in the enzyme active site pocket. Comparing among compounds **7**–**12**, it can be observed that the introduction of hydrogen bond forming groups (ketone or hydroxy always decreased inhibitory potency toward CYP11B2.With the intention of mimicking the natural substrate of CYP11B2, the unsaturated decalone analogue **17** was synthesized. However, only modest inhibition (52%) was observed.

The attempt to rigidify the core structure with a one-atom bridge (C or N) resulted in compounds **18**–**21**. When the bicyclo[2.2.1]heptane core was bearing the double bond, the resulting compound **18** showed only 36% of inhibition of CYP11B2. Nevertheless, the saturated compound **19** was much more potent (76%), but not selective. However, for the [3.2.1] aza-bicycle analogues a total loss of activity was observed probably as a consequence of the bulky bicycle core that was not tolerated.

CYP1A2 inhibition

With the aim of overcoming a common disadvantage of naphthalene or partially saturated naphthalene type CYP11B2 inhibitors — high CYP1A2 inhibition — this series of 3-pyridinyl substituted cycloaliphatic compounds, which are completely non-aromatic, were designed and synthesized. The five most potent CYP11B2 inhibitors with good CYP11B1 selectivity **2**, **4**, and **12**–**14** were selected to determine CYP1A2

inhibition (Table 2). As expected, all compounds exhibited significant improvement of selectivity toward CYP1A2 compared to the parent compounds **I**–**III**. It is striking that compounds **2**, **12** and **13** showed no inhibition at all at the concentration of 2 μM, whereas compound **14** with a decane cycle exhibited weak inhibition (17%). In contrast to the exo compounds **2** showing no CYP1A2 inhibition, the methylene analogue **4** showed moderate inhibition of 56%.This might be probably due to the exocyclic double bond being oxidized and activated by this monooxygenase to bind subsequently to the active site of the protein in a covalent manner. It is now clearly demonstrated that dearomatisation of the lipophilic core significantly increased the selectivity toward hepatic enzyme CYP1A2.

Table 2. Inhibition of human hepatic enzyme CYP1A2 by selected compounds.

Compd	% inhibition CYP1A2 [a]	Compd	% inhibition CYP1A2 [a]
I	98	**4**	56
II	80	**12**	0
III	73	**13**	0
2	0	**14**	17

[a] Recombinantly expressed enzyme from baculovirusinfected insect microsomes (Supersomes); inhibitor concentration, 2.0 μM; furafylline, 55% inhibition.

Conclusion

In this study, 21 analogues of 3-pyridinyl substituted aliphatic cycles were designed, synthesized and biologically evaluated as CYP11B2 inhibitors. Although the design conception was mainly focused on the improvement of CYP1A2 selectivity, these compounds exhibited potent inhibition of CYP11B2 and good selectivity over CYP11B1. Compounds **2**, **4**, **12**, **13** and **14** showed IC_{50} values ranging from 6 to 34 nM, whereby compound **4** is the most potent one (IC_{50} = 6 nM) and compound **12** is the most selective one (SF = 50). It has been demonstrated that an α-double bond in analogues with a H-bond forming groups in medium size cycles promoted inhibitory activity and selectivity, whereas deterioration was observed for 3-pyridinyl substituted medium size cycles without H-bond forming groups. Surprisingly, the presence of H-bond forming groups reduced inhibitory potency probably due to the binding mode alternation when these groups interacted with some amino acid residues. After removal of bridge bond, higher flexibility was obtained. Yet, this did not show significant influence. Moreover, the introduction of methylene as a potential interacting center increased not only CYP11B2 inhibition as expected, but also the undesired CYP1A2 inhibition. Bulk limitation was also observed. Flexible 8 to 10 membered medium or bicyclic rings are appropriate, whereas flexible 12-membered ring or rigid bicyclic analogues are too bulky to be tolerated.

Furthermore, the design concept employed in this study, i.e. Dearomatisation of the lipophilic core and destruction planarity configuration to reduce CYP1A2 inhibition, was proven successful. For the five most potent and selective CYP11B2 inhibitors in this study, the CYP1A2 inhibition was significantly reduced compared to their aromatic precursors. Among them, compounds **2**, **12** and **13** showed no inhibition at 2 μM. Since selectivity against hepatic CYP enzymes is always a key issue for safety reasons associated with drug

discovery, this task is occasionally challenging, especially in the CYP enzyme inhibitors field. The design concept demonstrated in this study would be helpful in the optimization of steroidogenic CYP inhibitors aimed at improvement of selectivity toward CYP1A2.

As it is known that fluorine compounds are more stable in vivo than hydroxy compounds, **R[1]** was sustained to be fluorine and the influence of substituents at the methylene bridge was further investigated (Table 3). Interestingly, the single ethyl group turned out to be the best, while twin alkyl substituted analogues (**24**–**25**) showed lower inhibitory potency than their single substituted analogues (**23**, **Ref. 2**). This is obviously due to the steric clashes with amino acids of the I-helix kink and the reduced flexibility of these ligands. Moreover, the similar activity of compound **27** (2-chloroethyl, IC_{50} = 756 nM) and compound **2** (*n*-Pr) and the total loss of activity for the 2-hydroxylethyl analogue (**26**) demonstrate the necessity of a hydrophobic side chain on the methylene bridge.

Experimental Section

Inhibition of CYP11B1 and CYP11B2

V79MZh cells expressing human CYP11B1 or CYP11B2 were incubated with [^{14}C]-11-deoxycorticosterone as substrate. The assay was performed as previously described.[22]

Inhibition of human hepatic CYP enzymes

The recombinantly expressed enzymes from baculovirus-infected insect microsomes (Supersomes) were used and the assay was performed according to the manufacturer's instructions (www.gentest.com).

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. High-resolution mass spectra were measured using an LTQ Orbitrap (Thermo Electron Corporation) with positive ESI. The purities of the final compounds were controlled by Surveyor®-LC-system. Purities were greater than 98%. 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). Ketones and precursors of ketones used as starting materials were commercially obtained (Aldrich, Acros, Merck, Alfa Aesar, etc.).

Method A: Triflation

To a solution of 2,6-di-*tert*-butyl-4-methylpyridine (7.23 mmol) and the corresponding ketone (6.57 mmol) in CH_2Cl_2 (20 mL) was added trifluoromethanesulfonic anhydride (7.16 mmol) dropwise at 0 °C under nitrogen. A white precipitate was formed soon. The reaction mixture was warmed to room temperature and further stirred for 4 h. The solvent was removed in vacuo, and the resulting residue was diluted with

petroleum ether (20 mL). After removal of the solid by filtration, the filtrate was concentrated in vacuo to give a crude product which was used in the next step without further purification.

Method B: Suzuki coupling

A suspension of enol triflate (4.56 mmol), pyridine-3-boronic acid (5.92 mmol), sodium carbonate (22.8 mmol) and tetrakis(triphenylphosphine)palladium (0) (0.23 mmol) in dimethoxyethanol (45 mL) and water (15 mL) was stirred at 90 °C under nitrogen for 2 h. The reaction mixture was cooled to room temperature naturally and diluted with water (10 mL). The aqueous layer was extracted with ethyl acetate (2 x 30 mL) and the combined organic layers were washed with brine twice and dried over MgSO4. After evaporation in vacuo, the resulting residue was purified by flash chromatography to afford the corresponding product.

Method C: Hydrogenation

A mixture of 3-pyridyl derivative with an α-double bond (0.50 mmol) and 5% Pd/C (45 mg) in methanol (15 mL) was flushed with hydrogen for 2 d at room temperature The mixture was filtered through a Celite cake and concentrated in vacuo to afford the corresponding product.

Method D: Sodium borohydride reduction

To a solution of a ketone (0.25 mmol) in methanol (5 mL) was added sodium borohydride (0.50 mmol) at room temperature The reaction was stirred for 4 h. After removal of MeOH in vacuo, the residue was eluted with water (5 mL) and then extracted with ethyl acetate (3 × 5 mL). The combined organic layers were washed with brine, dried over MgSO4 and concentrated in vacuo. The residue oil was purified by flash chromatography to give the title alcohol.

3-(Decahydro-naphthalen-2-yl)-pyridine (1): The title compound was synthesized according to Method C using 1a (300 mg, 1.41mmol) and 5% Pd/C (45 mg) in methanol (15 mL) to give a pale yellow oil (257 mg, 85%): R_f = 0.45 (EtOAc/PE,1:5); 1H-NMR (500 MHz, CDCl3): δ = 8.48 (d, *J* = 2.1 Hz, 1H), 8.42 (dd, *J* = 1.5, 4.8 Hz, 1H), 7.53 (dt, *J* = 1.9, 7.8 Hz, 1H), 7.20(dd, *J* = 4.8, 7.8 Hz, 1H), 2.57 (m, 1H),1.23–1.90 ppm (m, 16 H). ^{13}C-NMR (125 MHz, $CDCl_3$): δ = 149.0, 147.3, 142.8, 134.0, 123.2, 42.5, 36.4, 35.2, 33.5, 32.5, 32.2, 28.2, 27.0, 25.8, 20.9 ppm; IR: ν̃ = 2920, 2856, 1423, 1024, 802, 714, 622 cm^{-1}; MS (ESI) m/z = 216 $[M+H]^+$; HRMS: *m/z* $[M+H]^+$ calcd for $C_{15}H_{21}N$: 216.1752, found: 216.1744.

5-Pyridin-3-yl-3,3a,4,6a-tetrahydro-1H-pentalen-2-one (2): The title compound was synthesized according to Method B using 2a (290 mg, 1.07 mmol), pyridine-3-boronic acid (170 mg, 1.39 mmol), sodium carbonate (567 mg, 5.35 mmol) and tetrakis (triphenylphosphine)palladium (0) (61 mg, 0.05 mmol) in dimethoxyethanol (9 mL) and water (3 mL) to give a pale yellow oil (180 mg, 84%) after purification by flash chromatography (EtOAc/n-hexane,1:1): R_f = 0.19 (EtOAc/PE,2:1); ^{1}H-NMR (500 MHz, $CDCl_3$): δ = 8.67 (d, *J* = 1.9 Hz, 1H), 8.48 (dd, *J* = 0.9, 4.7 Hz, 1H), 7.68 (dt, *J* = 1.9, 7.9 Hz, 1H), 7.24 (dd, *J* = 4.7, 7.9 Hz, 1H) , 6.14 (m, 1H), 3.63 (m, 1H), 3.09–3.19 (m, 2H), 2.51–2.64 (m, 3H), 2.34 (d, *J* = 18.8 Hz, 1H), 2.09 ppm (dd, *J* = 7.4, 19.0 Hz, 1H); ^{13}C-NMR (125 MHz, $CDCl^3$): δ = 219.0, 148.6, 147.3, 139.3, 132.8, 131.4, 130.6, 123.2, 46.9, 44.9, 42.6, 40.3, 37.3 ppm; IR: ν̃ = 2904, 2360, 1733, 1567, 1486, 1424, 1411, 1324, 1158, 1119, 1023, 871, 813, 789, 705, 620 cm^{-1}; MS (ESI) *m/z* = 200 $[M+H]^+$.

5-Pyridin-3-yl-hexahydro-pentalen-2-one (3): The title compound was synthesized according to Method C using 2 (100 mg, 0.50 mmol) and 5% Pd/C (15% wt) in methanol (10 mL) to give a colorless oil (82 mg, 82%): R_f = 0.14 (EtOAc/PE,2:1); ^{1}H-NMR (500 MHz, $CDCl_3$): δ = 8.48 (s, 1H), 8.44 (d, *J* = 3.4 Hz, 1H), 7.52 (dt, *J* = 1.7, 7.9 Hz, 1H), 7.22 (dd, *J* = 4.8, 7.8 Hz, 1H), 3.13–3.20 (sept, *J* = 6.8 Hz, 1 H), 2.82–2.91 (m, 2H), 2.55–2.61 (m, 2 H , 2.51–2.46 (m, 2H), 2.12–2.17 (dd, *J* = 4.2, 19.3 Hz, 2H), 1.44–1.50 ppm (m, 2H); ^{13}C-NMR (125 MHz, $CDCl_3$): δ = 148.9, 147.7, 139.1, 134.0, 123.4, 44.6, 44.2, 41.9, 39.2 ppm; IR: ν̃ = 2949, 1733, 1575, 1427, 1169, 1025, 805, 716 cm^{-1}; MS (ESI) *m/z* = 202 [*M*+H]$^+$; HRMS: m/z [*M*+H]+ calcd for $C_{13}H_{16}NO$: 202.1232, found: 202.1217.

3-(5-Methylene-1,3a,4,5,6,6a-hexahydro-pentalen-2-yl)-pyridine (4): To a suspension of methyltriphenylphosphonium bromide (897 mg, 2.51 mmol) in THF (10 mL) was added a solution of n-BuLi in hexane (2.5 M, 1 mL, 2.50 mmol) at –78 °C under N_2. Subsequently, the reaction was stirred at room temperature for 3 h followed by cooling the reaction mixture to –78 °C. A solution of 2 (100 mg, 0.50 mmol) in THF (2 mL) was added slowly. The reaction was allowed to be warmed to room temperature and stirred for 24 h. A saturated aqueous solution of NH_4Cl (3 mL) was added to quench and the resulting mixture was extracted with ethyl acetate (3 x 15 mL). The combined organic layers were washed with brine (2 x 20 mL), dried over MgSO4 and concentrated in vacuo. The residue was purified by flash chromatography (AcOEt/n-hexane, 1:10) to give 4 as a colorless oil (72 mg, 73%): R_f = 0.44 (EtOAc/PE,1:1); ^{1}H-NMR (500 MHz, $CDCl_3$): δ = 8.66 (s, 1H), 8.44 (s, 1H), 7.66 (dt, *J* = 1.8, 7.8 Hz, 1H) , 7.21 (dd, *J* = 4.8, 7.8 Hz, 1H), 6.09 (q, *J* = 2.1 Hz, 1H), 4.79 (q, *J* = 1.8 Hz, 1H), 4.77 (q, *J* = 1.8 Hz, 1H), 3.42 (m, 1H) , 2.90–3.00 (m, 2H), 2.54–2.65 (m, 2H), 2.49 (m, 1H), 2.25 (m, 1H), 2.10 ppm (m, 1H); ^{13}C-NMR (125 MHz, $CDCl_3$): δ = 152.2, 148.0, 147.3, 138.1, 132.7, 132.0, 131.8, 123.1, 105.6, 50.7, 41.4, 40.5, 40.4, 38.2 ppm; IR: ν̃ = 2940, 2843, 1566, 1474, 1411, 1311, 1186, 1023, 880, 796, 706, 620 cm^{-1}; MS (ESI) *m/z* = 198 [*M*+H]$^+$; HRMS: *m/z* [*M*+H]$^+$ calcd for $C_{14}H_{16}N$: 198.1283, found: 198.1274.

5-Pyridin-3-yl-1,2,3,3a,4,6a-hexahydro-pentalen-2-ol (5): The title compound was synthesized according to Method D using 2 (50 mg, 0.25 mmol), sodium borohydride (19 mg, 0.50 mmol) in MeOH (5 mL) to give a pale yellow crystal (32 mg, 64%) after purification by flash chromatography (MeOH/CH_2Cl_2, 1:30). R_f = 0.18 (MeOH/ CH_2Cl_2,1:20); mp: 110–112 °C; ^{1}H-NMR (500 MHz, $CDCl_3$): δ = 8.37 (d, J = 1.6 Hz, 1H), 8.44 (dd, J = 1.1, 4.7 Hz, 1H), 7.68 (dt, J = 1.9, 7.9 Hz, 1H), 7.22 (ddd, J = 0.4, 4.7, 7.9 Hz, 1 H), 6.24 (q, *J* = 2.1 Hz, 1H), 4.25 (quin, *J* = 5.7 Hz, 1H), 3.32–3.38 (m, 1H), 3.01–3.07 (ddt, *J* = 2.1, 9.3, 16.2 Hz, 1H), 2.83–2.90 (tquin, J = 2.4, 9.0 Hz, 1H), 2.62–2.67 (dq, J = 2.4, 16.2 Hz, 1H), 2.23 (m, 1H), 2.14 (m, 1H), 1.75 (s, br, 1H), 1.52–1.62 ppm (m, 2H); ^{13}C-NMR (125 MHz, $CDCl_3$): δ = 148.1, 147.3, 137.3, 132.8, 132.4, 132.0, 123.2, 74.6, 49.7, 43.9, 40.7, 38.5 ppm; IR: ν̃ = 3221, 2957, 2929, 1444, , 1344, 1088, 1041, 810, 711, 629 cm^{-1}; MS (ESI) *m/z* = 202 [*M*+H]$^+$; HRMS: *m/z* [*M*+H]$^+$ calcd for $C_{13}H_{16}NO$: 202.1232, found: 202.1223.

5-Pyridin-3-yl-octahydro-pentalen-2-ol (6): The title compound was synthesized according to Method D using 3 (40 mg, 0.22 mmol), sodium borohydride (15 mg, 0.40 mmol) in MeOH (5 mL) to give a pale yellow semisolid (30 mg, 74%) after purification by flash chromatography (MeOH/CH_2Cl_2, 1:30). R_f = 0.17

(MeOH/CH_2Cl_2, 1:20); ^{1}H-NMR (500 MHz, $CDCl_3$): δ = 8.47 (d, *J* = 2.3 Hz, 1H), 8.39 (dd, *J* = 1.6, 4.8 Hz, 1 H), 7.55 (dt, *J* = 1.8, 7.9 Hz, 1H), 7.18 (dd, *J* = 4.8, 7.9 Hz, 1H), 4.38 (quin, *J* = 5.6 Hz, 1 H), 2.97–3.04 (sept, *J* = 6.1 Hz,, 1H), 2.53–2.75 (m, 2 H), 2.27–2.35 (m, 2H), 2.18 (s, br, 1H), 2.03–2.09 (m, 2H), 1.61–1.68 (m, 2H), 1.52–1.56 ppm (m, 2H); ^{13}C-NMR (125 MHz, $CDCl_3$): δ = 148.9, 147.2, 139.8, 134.3, 123.2, 76.5, 45.9, 42.7, 42.0 ppm; IR: ν˜ = 3318, 2936, 1578, 1426, 1097, 1027, 983, 713, 625 cm–1; MS (ESI) *m/z* = 204 [*M*+H]$^+$; HRMS: *m/z* [*M*+H]$^+$ calcd for $C_{13}H_{18}NO$: 204.1388, found: 204.1379.

5-Pyridin-3-yl-cyclooct-4-enone (7): The title compound was synthesized according to Method B using 7a (103 mg, 0.38 mmol), pyridine-3-boronic acid (60 mg, 0.49 mmol), sodium carbonate (200 mg, 1.89 mmol) and tetrakis(triphenylphosphine) palladium (0) (22 mg, 0.02 mmol) in dimethoxyethanol (6 mL) and water (2 mL) to give a pale yellow oil (50 mg, 65%) after purification by flash chromatography (EtOAc/n-hexane,1:5). R_f = 0.21 (EtOAc/PE, 2:1); ^{1}H-NMR (500 MHz, $CDCl_3$): δ = 8.58 (d, *J* = 1. 7 Hz, 1H), 8.46 (d, *J* = 4.7 Hz, 1H), 7.60 (dt, *J* = 1.7, 7.9 Hz, 1H), 7.21 (dd, *J* = 4.7, 7.9 Hz, 1H) , 6.06 (m, 1H), 2.60–2.65 (m, 6H), 2.51 (m, 2H), 1.71–1.76 ppm (m, 2H); ^{13}C-NMR (125 MHz, $CDCl_3$): δ = 212.3, 146.7, 145.9, 137.2, 136.5, 131.7, 127.4, 121.6, 45.6, 38.7, 28.3, 21.9, 21.8 ppm; IR: ν˜ = 2934, 1703, 1471, 1412, 1337, 1110, 892, 803, 712, 618 cm^{-1}; MS (ESI) *m/* = 202 [*M*+H]$^+$; HRMS: *m/z* [*M*+H]$^+$ calcd for $C1_3H_{16}NO$: 202.1232, found: 202.1224.

5-Pyridin-3-yl-cyclooctanone (8): The title compound was synthesized according to Method C using 7 (87 mg, 0.43 mmol) and 5% Pd/C (13 mg) in methanol (10 mL) to give a colorless oil (60 mg, 69%) after purification by flash chromatography (EtOAc/n-hexane,1:2). R_f = 0.21 (EtOAc/PE, 2:1); ^{1}H-NMR (500 MHz, $CDCl_3$): δ = 8.40 (d, *J* = 4.7 Hz, 1H); 8.37 (s, 1H), 7.40(dt, *J* = 1.9, 7.9 Hz, 1H), 7.17 (dd, *J* = 4.7, 7.9 Hz, 1H), 2.64–2.69 (m, 2H), 2.34–2.40 (m, 3H) , 2.13–2.21 (m, 2H), 1.81–1.90 ppm (m, 6H); ^{13}C-NMR (125 MHz, $CDCl_3$): δ = 217.1, 148.5, 147.4, 144.6, 134.0, 123.4, 42.0, 40.7, 36.1, 25.2 ppm; IR: ν˜ = 2930, 2858, 1696, 1446, 1258, 1209, 1024, 979, 849, 716 cm^{-1}; MS (ESI) *m/z* = 204 [*M*+H]$^+$; HRMS: *m/z* [*M*+H] calcd for $C_{13}H_{18}NO$: 204.1388, found: 204.1380.

5-Pyridin-3-yl-cyclooct-4-enol (9): The title compound was synthesized according to Method D using 7 (60 mg, 0.30 mmol), sodium borohydride (23 mg, 0.60 mmol) in MeOH (5 mL) to give a light yellow oil (30 mg, 49%) after flash chromatography (MeOH/CH_2Cl_2, 1:30). Rf = 0.14 (MeOH/CH_2Cl_2,1:20); ^{1}H-NMR (500 MHz, $CDCl_3$): δ = 8.61 (d, *J* = 2.2 Hz, 1H), 8.43 (dd, *J* = 1.5, 4.8 Hz, 1H), 7.64 (dt, *J* = 2.2, 7.9 Hz, 1H), 7.21 (dd, *J* = 4.8, 7.9 Hz, 1H), 6.08 (t, *J* = 8.2 Hz, 1H), 3.81 (m, 1H), 2.60 (t, *J* = 6.3 Hz, 2H), 2.41–2.49 (m, 1H), 2.24–2.30 (m, 1H), 1.98–2.04 (m, 1H), 1.92 (br, 1H), 1.82–1.87 (m, 1H), 1.74–1.79 (m, 2H), 1.59–1.70 ppm (m, 2H); ^{13}C-NMR (125 MHz, CDCl3): δ = 147.7, 147.1, 138.1, 137.0, 132.9, 129.3, 123.1, 72.8, 38.3, 36.2, 28.6, 24.9, 24.2 ppm; IR: ν˜ = 3329, 2924, 2853, 1472, 1414, 1077, 1041, 1023, 965, 803, 709 cm^{-1}; MS (ESI) *m/z* = 204 [*M*+H]$^+$; HRMS: *m/z* [*M*+H]$^+$ calcd for $C_{13}H_{18}NO$: 204.1388, found: 204.1380.

(endo/exo)-5-Pyridin-3-yl-cyclooctanol (10): The title compound was synthesized according to Method D using 8 (96 mg, 0.47 mmol), sodium borohydride (36 mg, 0.95 mmol) in MeOH (5 mL) to give a colorless oil (72 mg, 75%) after flash chromatography (MeOH/ CH_2Cl_2, 1:30). R_f = 0.24 (MeOH/ CH_2Cl_2, 1:20); ^{1}H-NMR (500 MHz, $CDCl_3$): δ = 8.41 (m, 2H), 7.46 (m, 1H), 7.19 (m, 1H), 3.86–4.04 (m, 1H) , 2.66–2.83 (m,

1H), 1.90–2.01 (m, 4H), 1.79–1.87 (m, 3H), 1.69–1.77 ppm (m, 6H); ^{13}C-NMR (125 MHz, CDCl3): δ = 148.7, 147.1, 144.9, 134.1, 123.4, 71.7, 71.6, 41.6, 40.3, 35.6, 35.2, 34.3, 22.6, 21.7 ppm; IR: ν˜ = 3362, 2923, 1577, 1425, 1053, 990, 796, 715, 624 cm^{-1}; MS (ESI) *m/z* = 206 [[*M*+H]$^+$; HRMS: *m/z* [[*M*+H]$^+$ calcd for $C_{13}H_{20}NO$: 206.1545, found: 206.1536.

3-Cyclooct-1-enyl-pyridine (11): The title compound was synthesized according to Method B using 11a (300 mg, 1.16 mmol), pyridine-3-boronic acid (186 mg, 1.51 mmol), sodium carbonate (614 mg, 5.81 mmol) and tetrakis(triphenylphosphine)palladium (0) (133 mg, 0. 11 mmol) in dimethoxyethanol (12 mL) and water (4 mL) to give a pale yellow oil (153 mg, 71%) after purification by flash chromatography (EtOAc/n-hexane,1:5). R_f = 0.38 (EtOAc/ PE, 1:5); ^{1}H-NMR (500 MHz, $CDCl_3$): δ = 8.65 (d, *J* = 2.1 Hz, 1H), 8.43 (dd, *J* = 1.3, 4.7 Hz, 1H), 7.66 (dt, *J* = 2.1, 7.9 Hz, 1H), 7.20 (dd, *J* = 4.7, 7.9 Hz, 1H), 6.04 (t, *J* = 8.3 Hz, 1H), 2.61 (t, *J* = 6.3 Hz, 2H), 2.30 (m, 2H), 1.57–1.66 (m, 4H),1.50–1.56 ppm (m, 4H); ^{13}C-NMR (125 MHz, $CDCl_3$): δ = 147.6, 147.4, 138.4, 137.4, 132.8, 129.8, 123.0, 29.8, 29.2, 28.2, 27.4, 26.8, 26.0 ppm; IR: ν˜ = 2922, 2850, 1471, 1412, 1021, 802, 709, 626 cm^{-1}; MS (ESI) *m/z* = 188 [*M*+H]$^+$; HRMS: *m/z* [*M*+H]$^+$ calcd for $C_{13}H_{18}N$: 188.1439, found: 188.1430.

3-Cyclooctyl-pyridine (12): The title compound was synthesized according to Method C using 11 (160 mg, 0.85 mmol) and 5% Pd/C (16 mg) in methanol (15 mL) to give a colorless oil (150 mg, 93%) after purification by flash chromatography (EtOAc/n-hexane,1:10). R_f = 0.22 (EtOAc/PE, 1:5); ^{1}H-NMR (500 MHz, $CDCl_3$): δ = 8.45 (d, *J* = 1.8 Hz, 1H), 8.40 (dd, 1H, *J* = 1.5, 4.8 Hz, 1H), 7.48 (dt, *J* = 4.8, 7.8 Hz, 1H), 7.18 (dd, *J* = 4.8, 7.8 Hz, 1H), 2.81–2.76(m, 1H), 1.73–1.86 (m, 6H), 1.58–1.68 ppm (m, 8H); ^{13}C-NMR (125 MHz, $CDCl_3$): δ = 149.0, 147.0, 145.1, 134.2, 123.3, 42.1, 34.2, 26.8, 26.2, 25.8 ppm; IR: ν˜ = 2920, 2851, 1574, 1476, 1446, 1423, 793, 715, 640, 613 cm^{-1}; MS (ESI) *m/z* = 190 [*M*+H]$^+$; HRMS: *m/z* [*M*+H]$^+$ calcd for $C_{13}H_{20}N$: 190.1596, found: 190.1587.

3-Cyclodec-1-enyl-pyridine (13): The title compound was synthesized according to Method B using 13a (410 mg, 1.43 mmol), pyridine-3-boronic acid (228 mg, 1.86 mmol), sodium carbonate (758 mg, 7.15 mmol) and tetrakis (triphenylphosphine)palladium (0) (83 mg, 0. 07 mmol) in dimethoxyethanol (15 mL) and water (5 mL) to give a pale yellow oil (205 mg, 67%) after purification by flash chromatography (EtOAc/n-hexane,1:20). Rf = 0.19 (EtOAc/PE,1:10); 1H-NMR (500 MHz, CDCl3): δ = 8.64 (d, J = 1.9 Hz, 1H), 8.46 (dd, J = 1.4, 4.7 Hz, 1H), 7.64 (dt, J = 1.9, 7.9 Hz, 1H), 7.21 (dd, J = 4.7, 7.9 Hz, 1H) , 5.70 (t, J = 8.4 Hz, 1H), 2.71 (t, J = 6.1 Hz, 2H) , 2.43 (q, J = 6.7 Hz, 2H), 1.67 (m, 2H) , 1.47–1.40 (m, 8H), 1.22 ppm (m, 2H); 13C-NMR (125 MHz, CDCl3): δ = 148.1, 147.8, 138.3, 136.8, 133.6, 131.5, 123.0, 27.6, 26.9, 26.6, 26.1, 25.7, 24.7, 21.2, 20.4 ppm; IR: ν˜ = 2921, 2850, 1474, 1444, 1411, 1022, 885, 804, 757, 712, 625 cm–1; MS (ESI) m/z = 216 [M+H]+; HRMS: m/z [M+H]+ calcd for $C_{15}H_{22}N$: 216.1752, found: 216.1744.

3-Cyclodecyl-pyridine (14): The title compound was synthesized according to Method C using 13 (63 mg, 0.29 mmol) and 5% Pd/C (10 mg) in methanol (6 mL) to give a colorless oil (48 mg, 75%) after purification by flash chromatography (EtOAc/n-hexane,1:10). R_f = 0.14 (EtOAc/PE, 1:5); ^{1}H-NMR (500 MHz, $CDCl_3$): δ = 8.45 (d, *J* = 2.0 Hz, 1H), 8.40 (dd, *J* = 1.6, 4.8 Hz, 1H), 7.50 (dt, *J* = 2.0, 7.9 Hz, 1H), 7.19 (dd, *J* = 4.8, 7.9 Hz, 1H), 2.97 (m, 1H),1.88–1.94 (m, 2H), 1.69–1.45 ppm (m, 16H); ^{13}C-NMR (125 MHz, $CDCl_3$): δ = 149.4,

147.2, 143.8, 134.6, 123.3, 39.6, 32.5, 25.1, 25.0, 23.9 ppm; IR: ν̃ = 2923, 2869, 1574, 1479, 1443, 1423, 1024, 806, 715, 632, 615 cm^{-1}; MS (ESI) *m/z* = 218 $[M+H]^+$; HRMS: *m/z* $[M+H]^+$ calcd for $C_{15}H_{24}N$: 218.1909, found: 218.1900.

3-Cyclododec-1-enyl-pyridine (15): The title compound was synthesized according to Method B using 15a (300 mg, 0.95 mmol), pyridine-3-boronic acid (153 mg, 1.24 mmol), sodium carbonate (506 mg, 4.77 mmol) and tetrakis(triphenylphosphine) palladium (0) (110 mg, 0. 09 mmol) in dimethoxyethanol (12 mL) and water (4 mL) to give a pale yellow oil (140 mg, 60%) after purification by flash chromatography (EtOAc/n-hexane,1:5). R_f = 0.38 (EtOAc/PE,1:5); ^{1}H-NMR (500 MHz, $CDCl_3$): δ = 8.54 (d, *J* = 2.1 Hz, 1H), 8.48 (dd, *J* = 1.6, 4.8 Hz, 1H), 7.56 (dt, *J* = 2.1, 7.9 Hz, 1H), 7.26 (dd, *J* = 4.8, 7.9 Hz, 1H), 5.86 (t, *J* = 8.1 Hz, 1H), 2.46 (t, *J* = 5.8 Hz, 2H), 2.00 (m, 2H),1.28–1.58 ppm (m, 16H); ^{13}C-NMR (125 MHz, $CDCl_3$): δ = 149.3, 147.4, 136.0, 135.5, 134.7, 132.1, 122.8, 36.5, 28.8, 27.3, 26.9, 25.9, 25.8, 24.0, 23.7, 23.5, 23.0 ppm; IR: ν̃ = 2925, 2855, 1461, 1408, 1022, 814, 716, 622 cm^{-1}; MS (ESI) *m/z* = 244 $[M+H]^{+;}$ HRMS: *m/z* $[M+H]^+$ calcd for $C_{17}H_{26}N$: 244.2065, found: 244.2057.

3-Cyclododecyl-pyridine (16): The title compound was synthesized according to Method C using 15 (30 mg, 0.22 mmol) and 5% Pd/C (10 mg) in methanol (6 mL) to give a colorless oil (26 mg, 87%) after purification by flash chromatography (EtOAc/n-hexane,1:10). R_f = 0.22 (EtOAc/PE, 1:5); ^{1}H-NMR (500 MHz, $CDCl_3$): δ = 8.43 (s, 1H), 8.41 (d, *J* = 4.7 Hz, 1H), 7.48 (d, *J* = 7.8, 1H), 7.19 (dd, *J* = 4.7, 7.8 Hz, 1H), 2.76(quin, *J* = 6.3 Hz, 1H), 1.79–1.85 (m, 2H), 1.26–1.50 ppm (m, 20H); ^{13}C-NMR (125 MHz, $CDCl_3$): δ = 149.6, 147.2, 142.5, 134.7, 123.2, 37.3, 31.2, 23.8, 23.7, 23.3, 23.2, 22.5 ppm; IR: ν̃ = 2929, 2848, 1575, 1469, 1424, 1026, 812, 733, 716, 609 cm^{-1}; MS (ESI) *m/z* = 246 $[M+H]^+$; HRMS: *m/z* $[M+H]^+$ calcd for $C_{17}H_{28}N$: 246.2222, found: 246.2213.

(±)-8a-Methyl-6-pyridin-3-yl-3,7,8,8a-tetrahydro-2H-naphthalen-1-one (17): The title compound was synthesized according to Method B using 17a (290 mg, 0.93 mmol), pyridine-3-boronic acid (149 mg, 1.21 mmol), sodium carbonate (492 mg, 4.65 mmol) and tetrakis(triphenylphosphine)palladium (0) (54 mg, 0.05 mmol) in dimethoxyethanol (12 mL) and water (4 mL) to give a pale yellow oil (200 mg, 90%) after purification by flash chromatography (EtOAc/n-hexane,1:2). R_f = 0.24 (EtOAc/PE,1:1); ^{1}H-NMR (500 MHz, $CDCl_3$): δ = 8.72 (d, *J* =1.8 Hz, 1H), 8.46 (dd, *J* = 1.0, 4.7 Hz, 1H), 7.72 (dt, *J* = 1.8, 8.0 Hz, 1H), 7.24 (dd, *J* = 4.7, 8.0 Hz, 1H), 6.54 (s, 1H), 5.86 (t, *J* = 4.4 Hz, 1H), 2.80 (ddd, *J* = 5.7, 7.2, 15.6 Hz, 1H), 2.66–2.73 (m, 1H), 2.50–2.62 (m, 3H), 2.38–2.44 (m, 1H), 2.10–2.14 (ddd, *J* = 2.2, 4.6, 13.4 Hz, 1H),1.63–1.69 ppm (m, 1H), 1.25 ppm (s, 3H); ^{13}C-NMR (125 MHz, $CDCl_3$): δ = 214.6, 148.2, 146.6, 139.8, 136.1, 132.5, 132.1, 125.7, 125.0, 123.1, 44.8, 35.4, 28.8, 24.3, 23.7, 22.6 ppm; IR: ν̃ = 2925, 2358, 1706, 1446, 1414, 1094, 1045, 896, 807, 707 cm^{-1}; MS (ESI) *m/z* = 240 $[M+H]^+$.

3-Bicyclo[2.2.1]hept-2-en-2-yl-pyridine (18): The title compound was synthesized according to Method B using 18a (410 mg, 1.69 mmol), pyridine-3-boronic acid (270 mg, 2.20 mmol), sodium carbonate (896 mg, 8.45 mmol) and tetrakis(triphenylphosphine)palladium (0) (98 mg, 0.08 mmol) in dimethoxyethanol (18 mL) and water (6 mL) to give a pale yellow oil (237 mg, 82%) after purification by flash chromatography (EtOAc/n-hexane,1:10). R_f = 0.21 (EtOAc/PE,1:5); ^{1}H-NMR (500 MHz, $CDCl_3$): δ = 8.66 (d, *J* = 2.1 Hz,

1H), 8.41 (d, *J* = 4.7 Hz, 1H), 7.65 (dt, *J* = 2.1, 7.9 Hz, 1H), 7.20 (dd, *J* = 4.7, 7.9 Hz, 1H) , 6.39 (d, *J* = 2.5 Hz, 1H), 3.32 (s, 1H), 3.02 (s, 1H), 1.76–1.83 (m, 2H), 1.54 (dt, *J* = 1.9, 8.2 Hz, 1H), 1.27 (d, *J* = 8.2 Hz, 1H), 1.09–1.18 ppm (m, 2H); ^{13}C-NMR (125 MHz, $CDCl_3$): δ = 146.7, 146.6, 144.8, 131.8, 131.8, 131.4, 123.3, 48.0, 43.2, 43.0, 26.5, 24.7 ppm; IR: ν˜ = 2962, 2871, 1684, 1585, 1418, 1301, 1024, 873, 798, 707 cm^{-1}; MS (ESI) *m/z* = 172 $[M+H]^+$.

(endo/exo)-3-Bicyclo[2.2.1]hept-2-yl-pyridine (19): The title compound was synthesized according to Method C using **18** (190 mg, 0.22 mmol) and 5% Pd/C (30 mg) in methanol (10 mL) to give a colorless oil (171 mg, 90%) after purification by flash chromatography (EtOAc/n-hexane,1:10). R_f = 0.22 (EtOAc/PE, 1:5); ^{1}H-NMR (500 MHz, $CDCl_3$): δ = 8.46 (d, *J* = 2.2 Hz, 1H), 8.40 (dd, *J* = 1.4, 4.7 Hz, 1H), 7.49 (d, *J* = 7.8 Hz, 1H), 7.20 (dd, *J* = 4.7, 7.8 Hz, 1H), 3.20 (m, 1H), 2.42 (t, *J* = 3.9 Hz, 1H), 2.35 (t, *J* = 4.3 Hz, 1H), 1.97–2.04 (tt, *J* = 3.9, 12.1 Hz,1H), 1.53–1.60 (m, 2H), 1.46 (m, 1H), 1.40 (ddd, *J* = 2.3, 5.7, 12.5 Hz, 1H), 1.28–1.34 (m, 1H), 1.18–1.24 ppm (m, 2H); ^{13}C-NMR (125 MHz, $CDCl_3$): δ = 150.1, 146.9, 138.8, 135.4, 122.9, 43.6, 42.2, 40.5, 37.4, 34.0, 30.0, 22.7 ppm; IR: *ν˜* = 2951, 2872, 1478, 1420, 1025, 802, 714, 615 cm^{-1}; MS (ESI) *m/z* = 174 $[M+H]^+$; HRMS: *m/z* $[M+H]^+$ calcd for $C_{12}H_{16}N$: 174.1283, found: 174.1273.

8-Methyl-3-pyridin-3-yl-8-aza-bicyclo[3.2.1]oct-2-ene (20): The title compound was synthesized according to Method B using **18a** (391 mg, 1.44 mmol), pyridine-3-boronic acid (230 mg, 1.87 mmol), sodium carbonate (763 mg, 7.2 mmol) and tetrakis(triphenylphosphine)palladium (0) (83 mg, 0.07 mmol) in dimethoxyethanol (18 mL) and water (6 mL) to give a pale yellow oil (201 mg, 70%) after purification by flash chromatography (EtOAc/n-hexane,1:10). R_f = 0.30 ($CHCl_2$/MeOH/Et_3N,100:10:1); ^{1}H-NMR (500 MHz, $CDCl_3$): δ = 8.63 (d, *J* = 1.9 Hz, 1H), 8.48 (dd, *J* = 1.3, 4.7 Hz, 1H), 7.64 (dt, *J* = 1.9, 7.9 Hz, 1H), 7.24 (dd, *J* = 4.7, 7.9 Hz, 1H), 6.32 (dt, *J* = 1.5, 5.6 Hz, 1H), 3.66 (tt, *J* = 5.8, 17.6 Hz, 2H), 2.98 (dd, *J* = 3.9, 17.6 Hz, 1H), 2.55 (s, 3H), 2.28–2.35 (m, 1H) , 2.18–2.25 (m, 2H), 2.02 (m, 1H), 1.72 ppm (m, 1H); ^{13}C-NMR (125 MHz, $CDCl_3$): δ = 148.6, 146.4, 134.6, 132.1, 130.5, 127.1, 123.2, 59.8, 58.4, 36.3, 33.1, 29.6, 29.1 ppm; IR: *ν˜* = 2941, 1415, 1260, 1156, 1030, 802, 708, 638 cm^{-1}; MS (ESI) *m/z* = 200 $[M]^+$.

(endo/exo)-8-Methyl-3-pyridin-3-yl-8-aza-bicyclo[3.2.1]octane (21): The title compound was synthesized according to Method C using **20** (100 mg, 0.50 mmol) and 5% Pd/C (15 mg) in methanol (10 mL) to give a colorless oil (92 mg, 91%) after purification by flash chromatography ($CHCl_2$/MeOH/Et_3N,100:10:1). R_f = 0.18 ($CHCl_2$/MeOH/Et_3N, 100:10:1); ^{1}H-NMR (500 MHz, $CDCl_3$): δ = 8.55 (d, *J* = 2.0 Hz, 1H), 8.40 (dd, *J* = 1.5, 4.7 Hz, 1H), 7.56 (dt, *J* = 1.7, 7.9 Hz, 1H), 7.18(dd, *J* = 4.7, 7.9 Hz, 1H), 3.20–3.27 (m, 2H), 3.07 (quin, *J* = 8.0 Hz, 1H), 2.43 (m, 2H), 2.24 (s, 3H), 2.03–2.12 (m, 2H), 1.57 (ddd, *J* = 1.3, 8.4, 13.9 Hz, 2H), 1.43 ppm (m, 2H); ^{13}C-NMR (125 MHz, $CDCl_3$): δ = 149.2, 147.1, 141.4, 134.5, 123.0, 59.5, 40.6, 38.3, 30.3, 28.1 ppm; IR: *ν˜* = 2935, 1575, 1478, 1451, 1332, 1024, 799, 714, 623 cm^{-1}; MS (ESI) *m/z* = 202 $[M]^+$

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Supporting Information

1. The synthetic procedures and characterization of all intermediates.

Method A: Triflation

To a solution of 2,6-di-*tert*-butyl-4-methylpyridine (7.23 mmol) and the corresponding ketone (6.57 mmol) in CH_2Cl_2 (20 mL) was added trifluoromethanesulfonic anhydride (7.16 mmol) dropwise at 0 °C under nitrogen. A white precipitate was formed soon. The reaction mixture was warmed to room temperature and further stirred for 4 h. The solvent was removed in vacuo, and the resulting residue was diluted with petroleum ether (20 mL). After removal of the solid by filtration, the filtrate was concentrated in vacuo to give a crude product which was used in the next step without further purification.

Method B: Suzuki coupling

A suspension of enol triflate (4.56 mmol), pyridine-3-boronic acid (5.92 mmol), sodium carbonate (22. 8 mmol) and tetrakis(triphenylphosphine)palladium (0) (0.23 mmol) in dimethoxyethanol (45 mL) and water (15 mL) was stirred at 90 °C under nitrogen for 2 h. The reaction mixture was cooled to room temperature naturally and diluted with water (10 mL). The aqueous layer was extracted with ethyl acetate (2 x 30 mL) and the combined organic layers were washed with brine twice and dried over $MgSO_4$. After evaporation in vacuo, the resulting residue was purified by flash chromatography to afford the corresponding product.

Trifluoro-methanesulfonic acid 3,4,4a,5,6,7,8,8a-octahydro-naphthalen-2-yl ester (1b): The title compound was synthesized according to Method A using a mixture of *cis* and *trans* of 2-decalone (1.00 g, 6.57 mmol), trifluoromethanesulfonic anhydride (1.20 mL, 7.16 mmol) and 2, 6-di-*tert*-butyl-4-methylpyridine (1.48 g, 7.23 mmol) in CH_2Cl_2 (20 mL) to give a yellow oil (1.30 g).

A isomer mixture of 3-(3,4,4a,5,6,7,8,8a-octahydro-naphthalen-2-yl)-pyridine and 3-(1,4,4a,5,6,7,8,8a-octahydro-naphthalen-2-yl)-pyridine (1a): The title compound was synthesized according to Method B using **1b** (1.29 g, 4.54 mmol), pyridine-3-boronic acid (0.73 g, 5.92 mmol), sodium carbonate (2.41 g, 22.8 mmol) and tetrakis (triphenylphosphine)palladium (0) (0.24 g, 0.21 mmol) in dimethoxyethanol (45 mL) and water (15 mL) to give a pale yellow oil (0.86 mg, 88%) after purification by flash chromatography (EtOAc / n-hexane,1:10): R_f = 0.45 (EtOAc/PE,1:5); MS (ESI) *m/z* = 214 $[M+H]^+$.

***cis*-Trifluoro-methanesulfonic acid 5-oxo-1,3a,4,5,6,6a-hexahydro-pentalen-2-yl ester (2a):** The title compound was synthesized according to Method A using *cis*-tetrahydropentalene-2,5(1*H*,3*H*)-dione (300 mg, 2.17 mmol), trifluoromethanesulfonic anhydride (0.36 mL, 2.17 mmol) and 2, 6-di-*tert*-butyl-4-methylpyridine (455 mg, 2.21 mmol) in CH_2Cl_2 (15 mL) to give a yellow oil (290 mg).

Cyclooctane-1,5-diol (7b): A solution of BH_3-THF complex in THF (1.0 M, 10 mL, 10.0 mmol) was stirred at 0 °C under N_2. A solution of 1,5-cyclooctadiene (1.00 g, 9.24 mmol) in THF (2 mL) was added slowly, and the resulting mixture was refluxed for 1 h. Upon cooling to room temperature, to the reaction mixture was added 3 M NaOH (2 mL), followed by 35% H_2O_2 (1.7 mL), which was added dropwise at a rate which caused the solution to reflux gently. The resulting mixture was allowed to cool for 30 min and

saturated with K_2CO_3. After separation, the aqueous layer was extracted with diethyl ether (2 × 8 mL). The combined organic layers were dried over $MgSO_4$ and concentrated in vacuo to give a colorless tar. The tar was dissolved in CH_2Cl_2 (60 mL), and PCC (9.24 g, 42.9 mmol) was added in three portions. The reaction mixture was refluxed for 2 d, and an additional PCC (4.70 g, 21.8 mmol) was added. After refluxing for 6 h, the reaction was cooled to room temperature and poured to a silica column, which was eluted with 1:1 diethyl ether/n-hexane. An offwhite semisolid (1.07 g, 82%) was obtained. ^{1}H-NMR (500 MHz, $CDCl_3$): δ = 2.49 (m, 8H), 2.13 ppm (m, 4H); ^{13}C-NMR (125 MHz, $CDCl_3$): δ = 213.0, 42.2, 22.1 ppm.

Trifluoro-methanesulfonic acid cyclooct-1-enyl ester (11a): The title compound was synthesized according to Method A using cyclooctanone (1.00 g, 7.87 mmol), trifluoromethanesulfonic anhydride (1.45 mL, 8.57 mmol) and 2, 6-di-*tert*-butyl-4-methylpyridine (1.78 g, 8.64 mmol) in CH_2Cl_2 (20 mL) to give a yellow oil (1.60 g).

Trifluoro-methanesulfonic acid cyclodec-1-enyl ester (13a): The title compound was synthesized according to Method A using cyclodecanone (300 mg, 1.94 mmol), trifluoromethanesulfonic anhydride (0.33 mL, 1.94 mmol) and 2, 6-di-*tert*-butyl-4-methylpyridine (407 mg, 1.98 mmol) in CH_2Cl_2 (15 mL) to give a yellow oil (410 mg).

Trifluoro-methanesulfonic acid cyclododec-1-enyl ester (15a): The title compound was synthesized according to Method A using cyclododecanone (1.00 g, 5.49 mmol), trifluoromethanesulfonic anhydride (1.01 mL, 5.98 mmol) and 2, 6-di-*tert*-butyl-4-methylpyridine (1.24 g, 6.03 mmol) in CH_2Cl_2 (20 mL) to give a yellow oil (1.71 g).

Trifluoro-methanesulfonic acid 4a-methyl-5-oxo-3,4,4a,5,6,7-hexahydro-naphthalen-2-yl ester (17a): The title compound was synthesized according to Method A using Wieland-Miescher ketone (200 mg, 1.12 mmol), trifluoromethanesulfonic anhydride (0.36 mL, 2.15 mmol) and 2, 6-di-*tert*-butyl-4-methylpyridine (481 mg, 2.34 mmol) in CH_2Cl_2 (8 mL) to give a yellow oil (294 mg).

Trifluoro-methanesulfonic acid bicyclo[2.2.1]hept-2-en-2-yl ester (18a): The title compound was synthesized according to Method A using norcamphor (500 mg, 4.54 mmol), trifluoromethanesulfonic anhydride (0.83 mL, 4.95 mmol) and 2, 6-di-*tert*-butyl-4-methylpyridine (1.03 g, 5.00 mmol) in CH_2Cl_2 (20 mL) to give a yellow oil (410 mg).

Trifluoro-methanesulfonic acid 8-methyl-8-aza-bicyclo[3.2.1]oct-2-en-3-yl ester (20a): To a solution of tropinone (500 mg, 4.54 mmol) in THF (20 mL) was added a solution of lithium hexamethyldisilazide in THF (1.0 M, 4.3 mL, 4.30 mmol) dropwise at –78 °C. The resulting mixture was stirred at the temperature for 30 min, and then was warmed to 0 °C. A solution of $PhNTf_2$ (1.54 g, 4.30 mmol) in THF (2 mL) was added. The reaction was stirred for 1 h at 0 °C, and subsequently stirred overnight at room temperature An aqueous solution of NH_4Cl (10 mL) was added to quench and the resulting mixture was separated. The aqueous layer was extracted with ethyl acetate (2 x 20 mL). The combined organic layers were washed with brine (3 x 30 mL), dried over $MgSO_4$ and concentrated in vacuo to give a red oil (391 mg), which was directly used for next step.

2. HPLC purity control of all final compounds.

The Surveyor®-LC-system consisted of a pump, an autosampler, and a PDA detector. Mass spectrometry was performed on a TSQ® Quantum (Thermo Electron Corporation, Dreieich, Germany). The triple quadrupole mass spectrometer was equipped with an electrospray interface (ESI). The system was operated by the standard software Xcalibur®.

A RP C18 NUCLEODUR® 100-5 (125 × 3 mm) column (Macherey-Nagel GmbH, Duehren, Germany) was used as stationary phase. All solvents were HPLC grade.

In a gradient run the percentage of acetonitrile (containing 0.1 % triflouro-acetic acid) in water was increased from an initial concentration of 3% at 0 min to 100% at 15 min and kept at 100% for 3 min.

The injection volume was 10 µl and flow rate was set to 350 µl/min. MS analysis was carried out at a spray voltage of 3800 V, a capillary temperature of 350 °C and a source CID of 10 V. Spectra were acquired in positive mode from 100 to 1000 m/z and full scan UV-mode. In some cases APC ionization had to be applied.

Compd	RT (min)	Purity [%]
1	7.98	99.9%
2	4.45	99.1%
3	3.55	99.1%
4	7.86	99.9%
5	5.10	98.1 %
6	5.14	99.3 %
7	4.46	99.0%
8	4.56	99.0%
9	7.72	99.9%
10	4.61	98.7%
11	7.81	98.4%
12	8.07	99.9%
13	9.01	98.0 %
14	9.31	99.3%
15	9.03	99.4%
16	8.99	99.9%
17	5.29	98.1%
18	5.04	99.9%
19	5.62	99.0%
20	8.81	98.3%
21	8.79	99.3%

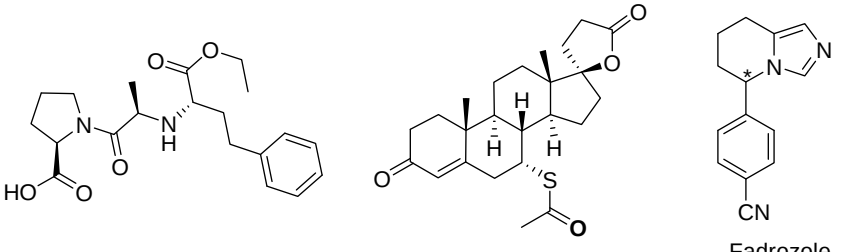
3.II. Novel Pyridyl or Isoquinolinyl Substituted Indolines and Indoles as Potent and Selective CYP11B2 Inhibitors

Introduction

Aldosterone is the important mineralocorticoid and regulates electrolyte and volume homeostasis after binding to mineralocorticoid receptors (MR). Normally, its secretion is controlled by the renin-angiotensin-aldosterone system (RAAS), adrenocorticotropic hormone (ACTH) and potassium concentration. However, in some pathological circumstances, the aldosterone levels are abnormally elevated. It has been demonstrated that high aldosterone levels are associated with severe cardiovascular diseases such as congestive heart failure, myocardial fibrosis, and certain forms of hypertension,[1] which can thereby lead to sudden death.

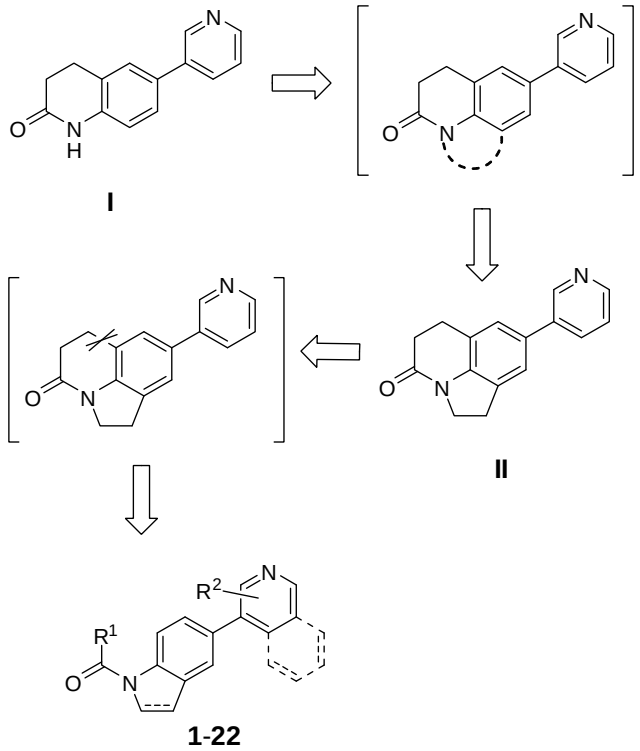
Aldosterone is biosynthesized from 11-deoxycorticosterone via three consecutive steps catalyzed by aldosterone synthase (CYP11B2), which is a mitochondrial cytochrome P450 enzyme located in the outer layer of adrenal cortex.[2] Therefore, it is rational to reduce high aldosterone levels through inhibition of CYP11B2, which has been proposed as a novel strategy for the treatment of aldosterone related heart diseases over a decade ago.[3] This therapy is believed to be superior to the treatment with angiotensin-converting-enzyme (ACE) inhibitors (such as enalapril, Chart 1) and MR antagonists (such as spironolactone, Chart 1) that are currently in clinical use. The reason for this is the "aldosterone escape" for ACE inhibitors[4] as a result of their long-term application and the outage of MR blockage at high aldosterone levels.[5] Recently, an in vivo study in rats has demonstrated that CYP11B2 inhibitors are effective in decreasing plasma aldosterone levels.[6] Experiments in rat heart failure models indicated that long-term administration of FAD286 (Chart 1), the *R*-enantiomer of fadrozole - a nonselective aromatase inhibitor also showing potent inhibition of CYP11B2 - improved both cardiac hemodynamics and cardiac function, which was more pronounced than that achieved by spironolactone.[7]

Chart 1. Structures of ACE inhibitor Enalapril, MR antagonist Spironolactone and aromatase inhibitor Fadrozole (*R*-enantiomer: FAD 286).



In the last few years, several classes of CYP11B2 inhibitors have been designed by our group[8] and others.[9] The same inhibitory mechanism as for aromatase (CYP19)[10], 17α-hydroxylase-17,20-lyase (CYP17) [11] and 11ß-hydroxylase (CYP11B1) inhibitors[12] - a *sp2* hybrid N reversibly coordinating to the heme iron - is employed in the design strategy. This is quite different from the inhibition mechanism of 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) inhibitors[13] and 5α-reductase inhibitors,[14] whose targets are also important enzymes in the steroid biosynthesis. This design strategy led to some extremely potent CYP11B2 inhibitors with IC_{50} values in a sub-nanomolar range.[8b,d,e] It is crucial that inhibition of other steroidogenic CYP enzymes should be avoided because they are essential for the biosynthesis of some vital steroid hormones, for example, CYP17 and CYP11A1 for androgens, CYP19 and CYP11A1 for estrogens, and CYP11B1, CYP21 and CYP11A1 for glucocorticoids. Inhibition of these enzymes will cause severe side effects. Due to the identical catalytic mechanism and the homology throughout the steroidogenic CYP enzyme family, especially to 11β-hydroxylase (CYP11B1), which shares more than 93% homology with CYP11B2, the selectivity is a real challenge. Nevertheless, it is possible to design highly selective compounds as it is shown by some CYP17[11e] and CYP11B2[8h,i] inhibitors. Often the key to high selectivity resides in small structural differences which can be caused by single small substituents,[11e] as it has been demonstrated in the design of dual inhibitors of CYP enzymes.[11d]

Chart 2. Inhibitor design concept.

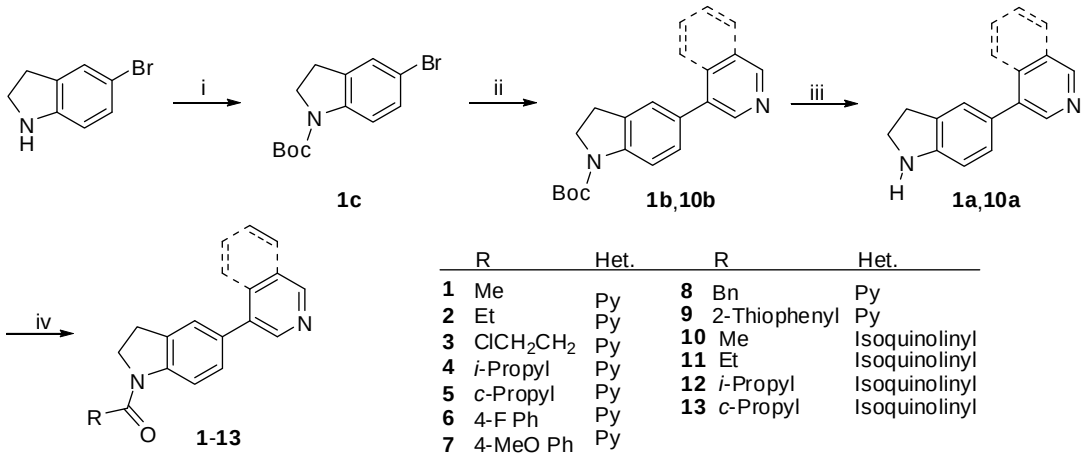


Results and Discussion

Chemistry

The synthetic routes are depicted in Schemes 1–3. Two consecutive key steps were employed for the synthesis of compounds **1**–**13**, **21** and **22**, which include Suzuki coupling with pyridin-3-yl boronic acid or

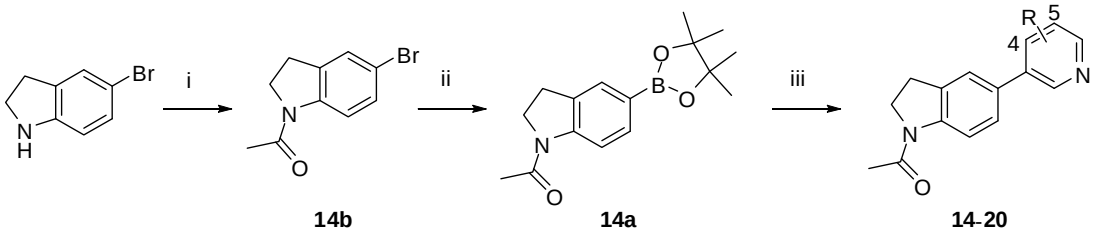
Scheme 1[a]



	R	Het.		R	Het.
1	Me	Py	**8**	Bn	Py
2	Et	Py	**9**	2-Thiophenyl	Py
3	$ClCH_2CH_2$	Py	**10**	Me	Isoquinolinyl
4	*i*-Propyl	Py	**11**	Et	Isoquinolinyl
5	*c*-Propyl	Py	**12**	*i*-Propyl	Isoquinolinyl
6	4-F Ph	Py	**13**	*c*-Propyl	Isoquinolinyl
7	4-MeO Ph	Py			

[a] Reagents and conditions: (i) Boc_2O, $NaHCO_3$, THF, H_2O, 0 °C to room temp; (ii) Method A: Pyridin-3-ylboronic acid or isoquinolin-4-ylboronic acid, $Ph(PPh_3)_4$, Na_2CO_3, DME, H_2O, 90 °C; (iii) Method B: CF_3COOH, CH_2Cl_2, 0 °C to room temp; (iv) Method C: RCOCl, Pyridine, THF, 0 °C to room temp.

Scheme 2[a]

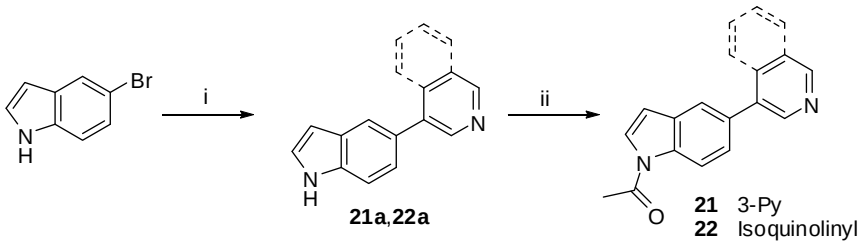


R		R		R	
14	5-F	**17**	5-Ac	**19**	5-Ph
15	5-CN	**18**	5-(1-OH-Et)	**20**	4-Me
16	5-MeO				

iv

[a] Reagents and conditions: (i) Method C: CH_3COCl, Pyridine, THF, 0 °C to room temp; (ii) Bis(pinacolato)diboron, $Pd(dppf)_2Cl_2$, KOAc, dioxane, 105 °C; (iii) Method A: R substituted 3-bromopyridine, $Ph(PPh_3)_4$, Na_2CO_3, DME, H_2O, 90 °C; (iv) $NaBH_4$, MeOH, 0 °C.

Scheme 3[a]



[a] Reagents and conditions: (i) Method A: Pyridin-3-yl boronic acid or isoquinolin-4-yl boronic acid, $Ph(PPh_3)_4$, Na_2CO_3, DME, H_2O, 90 °C; (ii) Method D: Ac_2O, Et_3N, DMAP, CH_2Cl_2, 60 °C.

isoquinolin-4-yl boronic acid to introduce the N-containing heterocycle and acylation in order to furnish various substituents on the indoline or indole core (Scheme 1 and 3). For the indoline analogues **1**–**13**, the amino group was primarily protected with Boc to ensure high yields in the Suzuki coupling, and was subsequently cleaved using trifluoroacetic acid before acylation was performed as the last step (Scheme 1).

With regard to the compounds with different substituents on the pyridyl moiety (**14**–**20**), boride **14a** was used as a common building block to achieve the last-stage diversification via Suzuki coupling with various substituted bromo-3-pyridines (Scheme 2). Compound **14a** was prepared by acetylation of 5-bromoindoline, and subsequent borylation with bis(pinacolato)diboron catalyzed by $Pd(dppf)_2Cl_2$.[15] A simple stoichiometric reduction of the ketone to the alcohol with sodium borohydride was performed to convert compound **17** into **18**.

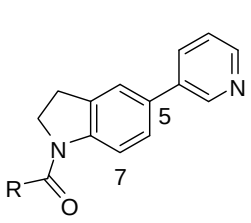
Inhibition of CYP11B2 and CYP11B1. The synthesized compounds were evaluated for their inhibitory potencies using V79 MZh cells expressing either human CYP11B1 or CYP11B2.[16] The results are presented with fadrozole as reference in tables 1–2.

After various acyl rests with different bulkiness and electrostatic potential were introduced into the indoline core (Table 1), steric hindrance was identified to be very detrimental for CYP11B2 inhibition. The Me compound **1** exhibited strong inhibition of CYP11B2 with an IC_{50} value of 60 nM and a good selectivity factor of 47. However, the ethyl analogue **2** showed a decrease in potency and selectivity (IC_{50} = 228 nM and SF = 21). Along with the increase in bulkiness of substituent R, the inhibitory potency of the corresponding compounds dropped dramatically to less than 30% at an inhibitor concentration of 500 nM (compounds **3**–**9**). Lipophilic or electronic properties of R as well as the existence of π-electron exerted only little effect. Intriguingly, benzyl substitution on the amido moiety led to a preference for CYP11B1 inhibition (**9**, 2.2 and 26% inhibition of CYP11B2 and CYP11B1, respectively).

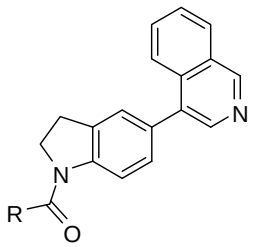
The modifications of the nitrogen-containing heterocycle were performed for improving inhibitory potency and selectivity. Positions adjacent to the N were left unsubstituted to avoid any obstacles for the N atom to coordinate with the heme iron. The 3-pyridyl moiety was fused with an additional benzene nucleus leading to an isoquinolinyl substituent. The resulting compounds **10**–**13**, which were furnished with methyl, ethyl, cyclopropyl or isopropyl at the amido moiety, exhibited substantial improvement regarding inhibitory activity and selectivity compared with the corresponding 3-pyridyl analogues (Table 1). It is obvious that the replacement of pyridyl by isoquinolinyl elevated the inhibitory potency by approximate 80-fold for the methyl (**10**) and ethyl (**11**) analogues to 0.7 and 2.8 nM, respectively. The inhibition of CYP11B2 by the cyclopropyl or isopropyl compounds **12** and **13** was also increased to 30 and 152 nM. Among these potent inhibitors, compounds **11** and **12** exhibited excellent selectivity over CYP11B1 with selectivity factors around 150. As expected, the influence of steric bulkiness was once again clearly observed in this series of compounds. With the increase in the substituents size from methyl and ethyl to cyclopropyl and isopropyl, the IC_{50} values rise from 0.7 nM to 152 nM.

Since methyl was discovered to be the most suitable group on the amido moiety, it was conserved when further substituents were introduced onto the 3-pyridyl ring. A wide range of the substituents were exploited for this further optimization, including electron donating (Me, MeO, and 1-OH-Et), electron withdrawing (F, CN, and Acetyl), and bulky aromatic (Ph) groups. In most cases, the resulting compounds exhibited significant amelioration of both potency and selectivity in comparison to the non-substituted pyridyl compound **1** (Table 2). It is apparent that electron withdrawing substituents cannot really improve the

Table 1. Inhibition of CYP11B1 and CYP11B2 by compounds **1**–**13**.



1 - 9



10 - 13

Compd	R	Inh.% @ 500 nM[c]		IC_{50} [nM][d]		SF[e]
		11B2[a]	11B1[b]	11B2[a]	11B1[b]	
1	Me	87	14	60	2848	47
2	Et	66	0.8	228	4737	21
3	$ClCH_2CH_2$	7.0	6.0	n.d.	n.d.	
4	*i*-Propyl	7.3	11	n.d.	n.d.	
5	*c*-Propyl	28	26	n.d.	n.d.	
6	4-F Ph	15	2.1	n.d.	n.d.	
7	4-MeO Ph	26	1.6	n.d.	n.d.	
8	2-Thiophenyl	14	4.9	n.d.	n.d.	
9	Bn	2.2	26	n.d.	n.d.	
10	Me	100	92	0.7	52	74
11	Et	100	39	2.8	516	184
12	*c*-Propyl	98	29	30	3723	124
13	*i*-Propyl	90	11	152	6093	40
Fadrozole	-			0.8	6.3	7.9

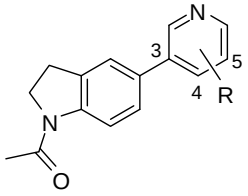
[a] Hamster fibroblasts expressing human CYP11B2; substrate: deoxycorticosterone, 100 nM. [b] Hamster fibroblasts expressing human CYP11B1; substrate: deoxycorticosterone, 100 nM. [c] Mean value of at least three experiments, standard deviation usually less than 10%. [d] Mean value of at least three experiments, relative standard deviation usually less than 25%. [e] SF (selectivity factor) = $IC_{50\ CYP11B2}$ / $IC_{50\ CYP11B1}$; n.d. = not determined.

inhibitory activity. When F was substituted at the 5-position of the pyridyl, the resulting compound **14** showed an IC_{50} value of 61 nM - a similar potency as the non-substituted analogue **1** - and an increase in selectivity by nearly 3-fold to 112. Whereas compound **17**, also with an electron withdrawing acetyl, exhibited an improved inhibitory activity of 36 nM, cyano substitution led to a loss of potency (compound **15**). The electron donating OMe group significantly increased CYP11B2 inhibition (**16**, 16 nM), and the 1-OH-Et compound (**18**) rendered similar potency as **1** (IC_{50} = 55 nM). Methyl substituted at the 4-position elevated the CYP11B2 inhibition to 2.2 nM (**20**), and also led to an excellent selectivity over CYP11B1 (SF = 166). It is interesting to observe that the bulky electron donating phenyl group increased inhibitory potency to 3 nM (**19**). This finding might be indicative of an additional hydrophobic pocket near the heme, which can be exploited to improve inhibitory activity.

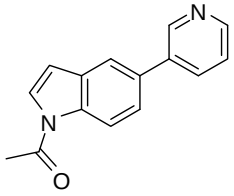
Furthermore, dehydrogenation of the indoline core resulting in the indole analogues **21** and **22** (Table 2)

showed an elevation of inhibitoty potency to 23 and 0.6 nM, respectively. However, no improvement of selectivity was observed (SFs around 60).

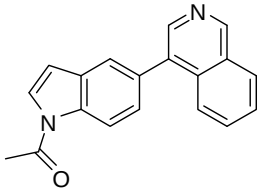
Table 2. Inhibition of CYP11B1 and CYP11B2 by compounds **14**–**22**.



14 - 20



21



22

Compd	R	Inh.% @ 500 nM[c]		IC_{50} [nM][d]		SF[e]
		11B2[a]	11B1[b]	11B2[a]	11B1[b]	
14	5-F	93	11	61	6812	112
15	5-CN	54	7.3	n.d.	n.d.	
16	5-MeO	100	48	16	803	50
17	5-Ac	97	24	36	3125	87
18	5-(1-OH-Et)	99	8.7	55	3756	68
19	5-Ph	100	74	3.0	160	53
20	4-Me	100	70	2.2	366	166
21		98	25	23	1379	60
22		100	97	0.6	34	57
Fadrozole	-			0.8	6.3	7.9

[a] Hamster fibroblasts expressing human CYP11B2; substrate: deoxycorticosterone, 100 nM. [b] Hamster fibroblasts expressing human CYP11B1; substrate: deoxycorticosterone, 100 nM. [c] Mean value of at least three experiments, standard deviation usually less than 10%. [d] Mean value of at least three experiments, relative standard deviation usually less than 25%. [e] SF (selectivity factor) = $IC_{50\ CYP11B2}$ / $IC_{50\ CYP11B1}$; n.d. = not determined.

Inhibition of Human CYP17 and CYP19. To investigate the selectivity profiles of this series of compounds, potent inhibitors (IC_{50} < 100 nM) were selected for further evaluation against CYP17 and CYP19. Inhibitory activity toward CYP17 was tested using the 50,000 sediments of *E.coli* homogenates coexpressing human CYP17 and cytochrome P450 reductase with an inhibitor concentration of 2 μM.[17a] As for CYP19 inhibition, microsomal preparations of human placenta were used with an inhibitor concentration of 500 nM.[18] All compounds exhibited no inhibition of CYP17 and CYP19 (data not shown).

Conclusion

A series of pyridyl or isoquinolinyl substituted indolines and indoles were designed by ligand-based approach: cleaving of the lactam ring and introduction of various substituents to the amido moiety resulted in very potent and selective CYP11B2 inhibitors, which might be promising for the treatment of aldosterone dependent cardiovascular diseases. It has been found that too bulky substituents at the amido group had a negative effect on inhibitory potency. The methyl group turned out to be the most suitable substituent. Modifications on the pyridyl by furnishing electron donating groups, such as methyl, methoxy and phenyl,

led to a significant increase of CYP11B2 inhibition. Interestingly, the highly potent inhibition exhibited by the phenyl substituted compound is indicative of an additional hydrophobic pocket near the heme. Another structure modification, the dehydrogenation of the indoline core also resulted in improved CYP11B2 inhibition, but had no real influence on selectivity. These novel potent CYP11B2 inhibitors exhibited no inhibition of other steroidogenic CYP enzymes, such as CYP17 and CYP19, indicating that the application of these compounds as CYP11B2 inhibitors can be expected to be safe regarding inhibition of other steroidogentic enzymes.

After a series of optimizations, highly potent and selective CYP11B2 inhibitors were identified especially compounds **11** and **20** (IC_{50} values around 2 nM and SFs around 170). These compounds can be considered as candidates for further evaluation in vivo.

Experimental Section

Biology

Inhibition of CYP11B1 and CYP11B2

V79MZh cells expressing human CYP11B1 or CYP11B2 were incubated with [1,2-^{3}H]-11-deoxycorticosterone as substrate and the inhibitor at different concentrations.. The assay was performed as previously described.[16]

CYP17 preparation and assay

Human CYP17 was used as 50,000 sediments of *E.coli*[17a] homogenates coexpressing the human enzyme and NADPH-P450 reductase, and the assay was performed using the method previously described, with progesterone as substrate and NADPH as cofactor.[17b]

CYP19 preparation and assay

Human CYP19 was obtained from microsomal preparations of human placenta and the assay was performed using the ^3H_2O-method as previously described and [1β-^{3}H]androstenedione as the substrate.[18]

Chemistry

General Method

Melting points were determined on a Mettler FP1 melting point apparatus and are uncorrected. ^{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. All coupling constants (*J*) are given in Hz. ESI (electrospray ionization) mass spectra were determined on a TSQ quantum (Thermo Electron Corporation) instrument. The purities of the final compounds were controlled by Surveyor-LC-system. Purities were greater than 95%. 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). Reagents and solvents were used as obtained from commercial suppliers without further purification.

Method A: Suzuki coupling

A suspension of bromo compound (1.0 equiv), boride (0.8–1.2 equiv), sodium carbonate (4.1–5.0 mmol) and tetrakis(triphenylphosphine)palladium (0) (0.05 equiv) in dimethoxyethane/water (3:1) was stirred at 90 °C under nitrogen for 2 h. The reaction mixture was cooled to room temperature slowly and then diluted with water. The aqueous layer was extracted with EtOAc and the combined organic layers were washed with brine twice and dried over $MgSO_4$. After evaporation in vacuo, the resulting residue was purified by flash chromatography to afford the corresponding product.

Method B: N-Boc Deprotection

Trifluoroacetic acid (9.0 equiv) was added to a solution of Boc-protected indoline (1.0 equiv) in anhydrous dichloromethane (25 mL) at 0 °C. The reaction was warmed to room temperature and stirred overnight. Subsequently, the reaction mixture was poured into a solution of saturated aqueous $NaHCO_3$ (25 mL). The resulting mixture was separated and the aqueous layer was extracted with dichloromethane (2 x 15 mL). The combined organic layers were washed with brine, dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by flash chromatography on silica gel (EtOAc/n-hexane, 1:50 to 1:1) to yield the deprotected product.

Method C: Acylation of Indoline

Acyl chloride (1.2 equiv) was added dropwise to a solution of substituted indoline (1.0 equiv) and pyridine (1.5 equiv) in anhydrous THF at 0 °C. The reaction was warmed to room temperature and stirred overnight. After water (5 mL) was added for quenching, the resulting mixture was separated. The aqueous layer was extracted with EtOAc (3 x 5 mL). The combined organic layers were washed with brine, dried over $MgSO_4$ and concentrated in vacuo. The resulting crude product was purified by flash chromatography on silica gel (EtOAc/n-hexane, 1:50 to 1:2).

Method D: Acetylation of Indole

To a stirred solution of 3-pyridinyl or isoquinolinyl substituted indole (1.0 equiv) in 1,2-dichloroethane (6 mL) was added triethylamine (1.5 equiv), acetic anhydride (3.9 equiv) and DMAP (0.2 equiv) in sequence under N_2. The solution was heated to 60 °C for 8h. Upon cooling to ambient temperature, the reaction was diluted with EtOAc (6 mL) and washed with a saturated solution of ammonium chloride, and the aqueous layer was extracted with EtOAc (3 x 10 mL).The combined organic layers were dried over $MgSO_4$ and concentrated in vacuo. The crude product was purified by flash column chromatography on silica gel (EtOAc/n-hexane, 1:50 to 1:2).

5-Pyridin-3-yl-2,3-dihydro-1*H*-indole (1a). The title compound was synthesized according to Method B using crude **1b** (1.40 g, 4.73 mmol) and trifluoroacetic acid (3.26 mL, 42.5 mmol) as a brown solid (0.93 g, 100%). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 3.11 (t, J = 8.4 Hz, 2H), 3.64 (t, J = 8.4 Hz, 2H), 6.71 (d, J = 8.1 Hz, 1H), 7.25 (m, 1H), 7.29 (ddd, J = 0.8, 4.9, 7.9 Hz, 1H), 7.35 (d, J = 1.2 Hz, 1H), 7.80 (dt, J = 2.1, 7.9 Hz, 1H), 8.49 (dd, J = 1.6, 4.8 Hz, 1H), 8.79 (dd, J = 0.6, 2.3 Hz, 1H).

1-Acetyl-5-pyridin-3-yl-2,3-dihydro-1*H*-indole (1). The title compound was synthesized according to

Method C using **1a** (80 mg, 0.41 mmol), acyl chloride (28 µL, 0.49 mmol) and pyridine (50 µL, 0.62 mmol) in anhydrous THF (3 mL). After flash column chromatography, recrystallization from THF gave yellow crystals (93 mg, 95%). mp 125–127 °C, R_f = 0.05 (EtOAc/n-hexane, 1:1). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 2.25 (s, 3H), 3.27 (t, *J* = 8.5 Hz, 2H), 4.12 (t, *J* = 8.5 Hz, 2H), 7.33 (dd, *J* = 4.8, 7.8 Hz, 1H), 7.42 (m, 2H), 7.83 (dt, *J* = 1.9, 7.8 Hz, 1H), 8.29 (d, *J* = 8.3 Hz, 1H), 8.55 (d, *J* = 4.5 Hz, 1H), 8.81 (d, *J* = 1.9 Hz, 1H). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 24.2, 28.0, 49.0, 117.3, 123.1, 123.5, 126.6, 132.1, 133.2, 134.0, 136.3, 143.0, 148.0, 148.1, 168.8. MS (ESI) *m/z* = 239 $[M+H]^+$.

1-Propanoyl-5-pyridin-3-yl-2,3-dihydro-1*H*-indole (2). The title compound was synthesized according to Method C using **1a** (200 mg, 1.02 mmol), propanoyl chloride (0.11 mL, 1.22 mmol) and pyridine (0.12 mL, 1.53 mmol) in anhydrous THF (3 mL). After flash column chromatography, recrystallization from THF gave offwhite needles (220 mg, 86%). mp 155–157 °C, R_f = 0.06 (EtOAc/n-hexane, 1:1). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 1.26 (t, *J* = 7.3 Hz, 3H), 2.48 (q, *J* = 7.3 Hz, 2H), 3.28 (t, *J* = 8.3 Hz, 2H), 4.11 (t, *J* = 8.3 Hz, 2H), 7.33 (dd, *J* = 4.9, 7.8 Hz, 1H), 7.41 (d, *J* = 3.4 Hz, 1H), 7.42 (s, 1H), 7.84 (dt, *J* = 2.0, 7.8 Hz, 1H), 8.34 (d, *J* = 8.0 Hz, 1H), 8.55 (d, *J* = 4.0 Hz,1H), 8.82 (d, *J* = 2.2 Hz, 1H). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 8.7, 28.0, 29.1, 48.0, 117.3, 123.1, 123.5, 126.7, 132.0, 133.0, 133.9, 136.3, 143.3,143.4, 148.0, 172.1. MS (ESI) *m/z* = 253 $[M+H]^+$.

4-(2,3-Dihydro-1*H*-indol-5-yl)isoquinoline (10a). The title compound was synthesized according to Method B using crude **10b** (1.97 g) and trifluoroacetic acid (2.43 mL, 31.8 mmol) and was obtained as yellow oil (0.58 g, 67% for three steps). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 3.14 (t, *J* = 8.4 Hz, 2H), 3.67 (t, *J* = 8.4 Hz, 2H), 6.78 (d, *J* = 7.9 Hz, 1H), 7.17 (dd, *J* = 1.7, 7.9 Hz, 1H), 7.27 (m, 1H), 7.61 (m, 1H), 7.66 (m, 1H), 8.01 (dd, *J* = 1.0, 8.4 Hz, 2H), 8.46 (s, 1H), 9.19 (s, 1H).

4-(1-Acetyl-2,3-dihydro-1*H*-indol-5-yl)isoquinoline (10). The title compound was synthesized according to Method C using **10a** (100 mg, 0.41 mmol), acetyl chloride (28 µL, 0.49 mmol) and pyridine (49 µL, 0.61 mmol) in anhydrous THF (5 mL). Purification by flash column chromatography twice gave a pale yellow solid (78 mg, 66%). mp 170–172 °C, R_f = 0.05 (EtOAc/n-hexane, 1:1). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 2.28 (s, 3H), 3.30 (t, *J* = 8.4 Hz, 2H), 4.16 (t, *J* = 8.4 Hz, 2H), 7.32 (s, 1H), 7.35 (d, *J* = 8.2 Hz, 1H), 7.62 (t, *J* = 7.4 Hz, 1H), 7.67 (t, *J* = 7.4 Hz, 1H), 7.93 (d, ^3J = 8.4 Hz, 1H), 8.03 (d, *J* = 7.9 Hz, 1H), 8.35 (d, *J* = 8.2 Hz, 1H), 8.46 (s, 1H), 9.24 (s, 1H).^{13}C-NMR (125 MHz, $CDCl_3$): δ 24.2, 28.0, 49.0, 116.9, 124.8, 126.1, 127.2, 127.9, 128.4, 129.6, 130.6, 131.7, 132.2, 133.2, 134.4, 142.6, 142.9, 151.6, 168.8. MS (ESI) *m/z* = 289 $[M+H]^+$.

4-(1-Propanoyl-2,3-dihydro-1*H*-indol-5-yl)isoquinoline (11). The title compound was synthesized according to Method C using **10a** (80 mg, 0.32 mmol), propanoyl chloride (34 µL, 0.39 mmol) and pyridine (39 µL, 0.49 mmol) in anhydrous THF (3 mL). Purification by flash column chromatography was performed twice and gave a pale yellow solid (65 mg, 67%). mp 153–155 °C, R_f = 0.12 (EtOAc/n-hexane, 1:1). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 1.28 (t, *J* = 7.3 Hz, 3H), 2.51 (q, *J* = 7.3 Hz, 2H), 3.30 (t, *J* = 8.4 Hz, 2H), 4.14 (t, *J* = 8.4 Hz, 2H), 7.32 (s, 1H), 7.35 (d, *J* = 8.3 Hz, 1H), 7.62 (t, *J* = 7.3 Hz, 1H), 7.67 (t, *J* = 7.3 Hz, 1H), 7.93 (d, *J* = 8.4 Hz, 1H), 8.03 (d, *J* = 8.0 Hz, 1H), 8.39 (t, *J* = 8.1 Hz, 1H), 8.46 (s, 1H), 9.23 (s, 1H).^{13}C-

NMR (125 MHz, $CDCl_3$): δ 8.7, 28.1, 29.2, 48.1, 116.8, 124.8, 126.1, 127.1, 127.9, 128.4, 129.6, 130.5, 131.5, 132.1, 133.1, 134.3, 136.3, 143.1, 151.7, 172.2. MS (ESI) *m/z* = 303 $[M+H]^+$.

4-[1-(Cyclopropylcarbonyl)-2,3-dihydro-1*H*-indol-5-yl]isoquinoline (12). The title compound was synthesized according to Method C using **10a** (80 mg, 0.32 mmol), cyclopropanecarbonyl chloride (36 µL, 0.40 mmol) and pyridine (39 µL, 0.49 mmol) in anhydrous THF (3 mL). Purification by flash column chromatography was performed twice and gave a pale yellow solid (77 mg, 76%). mp 159–162 °C, R_f = 0.11 (EtOAc/n-nexane, 1:1). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 0.93 (m, 2H), 1.17 (m, 2H), 1.81 (s, br, 1H), 3.33 (s, br, 2H), 4.37 (s, br, 2H), 7.34 (m, 2H), 7.62–7.70 (m, 2H), 7.94 (d, *J* = 8.1 Hz, 1H), 8.04 (d, *J* = 7.7 Hz, 1H), 8.32 (s, br, 1H), 8.47 (s, 1H), 9.24 (s, 1H).^{13}C-NMR (125 MHz, $CDCl_3$): δ 8.3, 13.7, 27.9, 48.3, 116.8, 124.8, 126.1, 127.2, 127.9, 128.4, 129.6, 130.6, 131.7, 131.9, 133.3, 134.4, 142.4, 143.2, 151.5, 172. MS (ESI) *m/z* = 315 $[M+H]^+$.

4-[1-(2-Methylpropanoyl)-2,3-dihydro-1*H*-indol-5-yl]isoquinoline (13). The title compound was synthesized according to Method C using **10a** (80 mg, 0.32 mmol), isobutyryl chloride (36 µL, 0.40 mmol) and pyridine (39 µL, 0.49 mmol) in anhydrous THF (3 mL). Purification by flash column chromatography was performed twice and gave a pale yellow solid (70 mg, 69 %). mp 149–151 °C, R_f = 0.12 (EtOAc/n-hexane, 1:1). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 1.28 (d, *J* = 6.7 Hz, 6H), 2.84 (m, 1H), 3.31 (t, *J* = 7.9 Hz, 2H), 4.24 (t, *J* = 8.5 Hz, 2H), 7.35 (m, 2H), 7.66 (m, 1H), 7.72 (m, 1H), 7.97 (d, *J* = 8.1 Hz, 1H), 8.07 (d, *J* = 7.8 Hz, 1H), 8.42 (s, *J* = 7.8 Hz, 1H), 8.47 (s, 1H), 9.26 (s, 1H).^{13}C-NMR (125 MHz, $CDCl_3$): δ 19.2, 28.1, 33.5. 48.1, 117.2, 124.8, 126.0, 127.1, 127.9, 128.4, 129.6, 130.5, 131.8, 132.2, 133.1, 134.3, 142.8, 143.2, 151.7, 175.8. MS (ESI) *m/z* = 317 $[M+H]^+$.

1-[5-(4,4,5,5-Tetramethyl-[1,3,2]dioxaborolan-2-yl)-2,3-dihydro-indol-1-yl]-ethanone (14a). To a solution of **14b** (1.86 g, 7.75 mmol) in dioxane (31 mL) was added bis(pinacolato)diboron (3.93 g, 15.5 mmol), $Pd(dPPf)_2Cl_2$ (0.57 g, 0.78 mmol) and anhydrous potassium acetate (3.80 g, 38.7 mmol) under N_2. The reaction was heated at 105 °C for 2 h. Upon cooling to room temperature, water (20 mL) was added and the resulting mixture was extracted with EtOAc (3 x 15 mL). The combined organic layers were washed with brine, dried over $MgSO_4$ and concentrated in vacuo. The crude product was purified by flash column chromatography on silica gel (EtOAc/n-hexane, 1:50 to 1:2) to yield a pale yellow solid (2.08 g, 94%). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 1.34 (s, 12H), 2.32 (s, 3H), 3.18 (t, *J* = 8.4 Hz, 2H), 4.05 (t, *J* = 8.5 Hz, 2H), 7.62 (s, 1H), 7.67 (d, *J* = 8.0 Hz, 1H), 8.19 (d, *J* = 8.1 Hz, 1H).

1-Acetyl-5-(5-fluoropyridin-3-yl)-2,3-dihydro-1*H*-indole (14). The title compound was synthesized according to Method A using **14a** (150 mg, 0.52 mmol), 3-bromo-5-fluoropyridine (111 mg, 0.63 mmol), sodium carbonate (277 mg, 2.61 mmol) and tetrakis(triphenylphosphine)palladium (0) (30 mg, 0. 03 mmol) in dimethoxyethane (9 mL) and water (3 mL). The crude product was purified by flash column chromatography on silica gel ($MeOH/CH_2Cl_2$, 0 to 2%) and crystallization from acetone to yield colorless crystals (108 mg, 81%). mp 178–180 °C, R_f = 0.26 ($MeOH/CH_2Cl_2$, 1:20). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 2.26 (s, 3H), 3.28 (t, *J* = 8.4 Hz, 2H), 4.13 (t, *J* = 8.5 Hz, 2H), 7.40 (m, 2H), 7.56 (dt, *J* = 2.0, 9.6 Hz, 1H), 8.30 (d, *J* = 8.2 Hz,1H), 8.41 (d, *J* = 1.8 Hz,1H), 8.64 (s, 1H).^{13}C-NMR (125 MHz, $CDCl_3$): δ 24.2, 27.9,

49.0, 117.4, 118.1, 120.7 (d, $^{2}J_{C,F}$ = 18.6 Hz), 123.2, 126.8, 131.5, 132.3, 136.0 (d, $^{2}J_{C,F}$ = 23.3 Hz), 138.1 (d, $^{4}J_{C,F}$ = 3.6 Hz), 143.6 (d, $^{4}J_{C,F}$ = 3.2 Hz), 159.7 (d, $^{1}J_{C,F}$ = 257 Hz), 168.9. MS (ESI) *m/z* = 257 $[M+H]^{+}$.

5-(1-Acetyl-2,3-dihydro-1*H*-indol-5-yl)pyridine-3-carbonitrile (15). The title compound was synthesized according to Method A using **14a** (150 mg, 0.52 mmol), 5-bromonicotinonitrile (118 mg, 0.63 mmol), sodium carbonate (277 mg, 2.61 mmol) and tetrakis(triphenylphosphine)palladium (0) (30 mg, 0. 03 mmol) in dimethoxyethane (9 mL) and water (3 mL). The crude product was purified by flash column chromatography on silica gel (MeOH/CH_2Cl_2, 0 to 2%) and crystallization from acetone to yield a white solid (123 mg, 90%). mp 206–207 °C, R_f = 0.31 (MeOH/CH_2Cl_2, 1:20). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 2.27 (s, 3H), 3.30 (t, *J* = 8.4 Hz, 2H), 4.15 (t, *J* = 8.5 Hz, 2H), 7.40 (m, 2H), 8.10 (t, *J* = 2.1 Hz, 1H), 8.34 (d, *J* = 8.3 Hz, 1H), 8.80 (d, *J* = 1.5 Hz, 1H), 9.00 (d, *J* = 2.2 Hz, 1H).^{13}C-NMR (125 MHz, $CDCl_3$): δ 24.2, 27.9, 49.0, 110.1, 116.5, 117.6, 123.1, 126.9, 130.4, 132.7, 136.8, 136.9, 144.1, 150.0, 151.0, 169.0. MS (ESI) *m/z* = 264 $[M+H]^{+}$.

1-Acetyl-5-(5-methoxypyridin-3-yl)-2,3-dihydro-1*H*-indole (16). The title compound was synthesized according to Method A using **14a** (150 mg, 0.52 mmol), 3-bromo-5-methoxypyridine (122 mg, 0.63 mmol), sodium carbonate (277 mg, 2.61 mmol) and tetrakis(triphenylphosphine) palladium (0) (30 mg, 0. 03 mmol) in dimethoxyethane (9 mL) and water (3 mL). The crude product was purified by flash column chromatography on silica gel (MeOH/CH_2Cl_2, 0 to 2%) and crystallization from acetone to yield colorless crystals (99 mg, 71%). mp 166–168 °C, R_f = 0.22 (MeOH/CH_2Cl_2, 1:20). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 2.25 (s, 3H), 3.27 (t, *J* = 8.5 Hz, 2H), 3.92 (s, 3H), 4.12 (t, *J* = 8.5 Hz, 2H), 7.35 (t, *J* = 2.1 Hz,1H), 7.40 (m, 2H), 8.26 (d, *J* = 2.6 Hz,1H), 8.29 (d, *J* = 8.3 Hz,1H), 8.43 (d, *J* = 1.8 Hz,1H).^{13}C-NMR (125 MHz, $CDCl_3$): δ 24.2, 28.0, 49.0, 55.7, 117.3, 119.1, 123.2, 126.8, 132.1, 132.8, 135.2, 137.3, 140.0, 143.2, 155.9, 168.8. MS (ESI) *m/z* = 269 $[M+H]^{+}$.

1-[5-(1-Acetyl-2,3-dihydro-1*H*-indol-5-yl)pyridin-3-yl]ethanone (17). The title compound was synthesized according to Method A using **14a** (300 mg, 1.04 mmol), 3-acetyl-5-bromopyridine (259 mg, 1.26 mmol), sodium carbonate (551 mg, 5.20 mmol) and tetrakis(triphenylphosphine)palladium (0) (60 mg, 0. 05 mmol) in dimethoxyethane (15 mL) and water (5 mL). The crude product was purified by flash column chromatography on silica gel (MeOH/CH_2Cl_2, 0 to 1:50) and crystallization from acetone to yield colorless crystals (250 mg, 86%). mp 152–154 °C, R_f = 0.23 (MeOH/CH_2Cl_2, 1:20). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 2.26 (s, 3H), 2.69 (s, 3H), 3.29 (t, *J* = 8.5 Hz, 2H), 4.13 (t, *J* = 8.5 Hz, 2H), 7.45 (m, 2H), 8.32 (t, *J* = 8.3 Hz,1H), 8.38 (t, *J* = 2.1 Hz,1H), 8.98 (d, *J* = 2.0 Hz,1H), 9.01 (s, 1H).^{13}C-NMR (125 MHz, $CDCl_3$): δ 24.2, 26.9, 27.9, 49.0, 117.4, 123.2, 126.7, 131.8, 132.3, 132.4, 133.2, 136.6, 143.6, 147.8, 151.4, 168.9, 196.7. MS (ESI) *m/z* = 281 $[M+H]^{+}$.

1-[5-(1-Acetyl-2,3-dihydro-1*H*-indol-5-yl)pyridin-3-yl]ethanol (18). To a solution of **17** (120 mg, 0.43 mmol) in methanol (5 mL) was added sodium borohydride (32 mg, 0.86 mmol) at 0 °C. The mixture was stirred at the same temperature for 1 h before removal of the solvent. The residue was diluted with water (5 ml) and then extracted with EtOAc (2 × 5 mL). The combined organic layers were washed with brine, dried over $MgSO_4$ and concentrated in vacuo.The residue was purified by flash column chromatography on silica

gel (MeOH/CH_2Cl_2, 0 to 5%) and crystallization from acetone to yield a pale yellow solid (120 mg, 98%). mp 179–181 °C, R_f = 0.06 (MeOH/CH_2Cl_2, 1:20). ^{1}H-NMR (500 MHz, MeOD-d_4): δ 1.53 (s, J = 6.5 Hz, 3H), 2.67 (s, 3H), 3.29 (d, J = 5.3 Hz, 2H), 4.19 (d, J = 3.8 Hz, 2H), 4.98 (q, J = 6.5 Hz, 1H), 7.49 (s, 1H), 7.55 (s, 1H), 8.05 (s, 1H), 8.20 (s, 1H), 8.49 (s, 1H), 8.66 (s, 1H).^{13}C-NMR (125 MHz, MeOD-d_4): δ 24.0, 25.5, 28.8, 50.3, 68.5, 118.4, 124.5, 127.3, 133.2, 134.4, 134.8, 138.0, 143.9, 144.3, 146.1, 146.8, 171.7. MS (ESI) m/z = 283 [M+H] $^+$.

1-Acetyl-5-(5-phenylpyridin-3-yl)-2,3-dihydro-1*H*-indole (19). The title compound was synthesized according to Method A using **14a** (150 mg, 0.52 mmol), 3-bromo-5-phenylpyridine (153 mg, 0.63 mmol), sodium carbonate (277 mg, 2.61 mmol) and tetrakis(triphenylphosphine)palladium (0) (30 mg, 0. 03 mmol) in dimethoxyethane (9 mL) and water (3 mL). The crude product was purified by flash column chromatography on silica gel (MeOH/CH_2Cl_2, 0 to 2%) and crystallization from acetone to yield pale yellow crystals (137 mg, 84%). mp 168–170 °C, R_f = 0.24 (MeOH/CH_2Cl_2, 1:20). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 2.26 (s, 3H), 3.29 (t, J = 8.4 Hz, 2H), 4.13 (t, J = 8.4 Hz, 2H), 7.42–7.52 (m, 5H), 7.64 (m, 2H), 8.03 (d, J = 2.0 Hz,1H), 8.32 (d, J = 8.3 Hz,1H), 8.79 (m, 2H).^{13}C-NMR (125 MHz, $CDCl_3$): δ 24.2, 28.0, 49.0, 117.4, 123.2, 126.8, 127.2, 128.3, 129.1, 132.2, 132.8, 132.9, 136.5, 136.8, 137.6, 143.3, 146.2, 146.3, 168.8. MS (ESI) m/z = 315 [M+H] $^+$.

1-Acetyl-5-(4-methylpyridin-3-yl)-2,3-dihydro-1*H*-indole (20). The title compound was synthesized according to Method A using **14a** (150 mg, 0.52 mmol), 3-bromo-4-methylpyridine (70 µL, 0.63 mmol), sodium carbonate (277 mg, 2.61 mmol) and tetrakis(triphenylphosphine)palladium (0) (30 mg, 0. 03 mmol) in dimethoxyethane (9 mL) and water (3 mL). The crude product was purified by flash column chromatography on silica gel (MeOH/CH_2Cl_2, 0 to 2%) and crystallization from acetone to yield pale yellow crystals (85 mg, 65%). mp 180–182 °C, R_f = 0.50 (MeOH/CH_2Cl_2 1:20). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 2.25 (s, 3H), 2.29 (s, 3H), 3.26 (t, J = 8.5 Hz, 2H), 4.12 (t, J = 8.5 Hz, 2H), 7.12–7.18 (m, 3H), 8.27 (d, J = 8.2 Hz,1H), 8.41 (m, 2H).^{13}C-NMR (125 MHz, $CDCl_3$): δ 19.9, 24.1, 28.0, 48.9, 116.7, 125.2, 125.3, 128.8, 131.5, 133.1, 137.6, 142.5, 144.8, 147.8, 149.7, 168.8. MS (ESI) m/z = 253 [M+H] $^+$.

5-Pyridin-3-yl-1*H*-indole (21a). The title compound was synthesized according to Method A using crude 5-bromoindole (250 mg, 1.26 mmol), pyridin-3-ylboronic acid (186 mg, 1.51 mmol), sodium carbonate (667 mg, 6.30 mmol) and tetrakis(triphenylphosphine)palladium (0) (73 mg, 0.06 mmol) in dimethoxyethane (30 mL) and water (10 mL). The crude product was purified by flash column chromatography on silica gel (EtOAc/n-hexane, 1:100 to 1:2) to yield a pale yellow solid (202 mg, 82%). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 7.37 (ddd, J = 0.8, 4.8, 7.9 Hz, 1H), 7.41 (m, 1H), 7.49 (m, 2H), 7.59 (m, 2H), 7.88 (dt, J = 2.0, 7.9 Hz, 1H), 8.60 (dd, J = 1.6, 4.8 Hz, 1H), 8.86 (dd, J = 0.6, 2.4 Hz, 1H).

1-Acetyl-5-pyridin-3-yl-1*H*-indole (21). The title compound was synthesized according to Method D using **21a** (163 mg, 0.84 mmo), triethylamine (0.17 ml, 1.26 mmol), acetic anhydride (0.30 ml, 3.28 mmol) and DMAP (20 mg, 0.16 mmol) and was obtained as pale yellow crystals (100 mg, 50%). mp 138–140 °C, R_f = 0.16 (EtOAc/n-hexane, 1:1). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 2.67 (s, 3H), 6.71 (d, J = 3.8 Hz, 1H), 7.38 (dd, J = 4.8, 7.9 Hz, 1H), 7.48 (d, J = 3.7 Hz, 1H), 7.57 (dd, J = 1.8, 8.5 Hz, 1H), 7.77 (d, J = 1.7 Hz, 1H),

7.93 (dt, J = 1.8, 7.9 Hz, 1H), 8.54 (d, J = 8.5 Hz, 1H), 8.59 (dd, J = 1.5, 4.8 Hz, 1H), 8.90 (d, J = 1.8 Hz, 1H). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 23.9, 109.2, 117.1, 119.4, 123.6, 124.4, 126.1, 131.1, 133.4, 134.7, 135.4, 137.0, 148.0, 148.3, 168.5. MS (ESI) m/z = 237 $[M+H]^+$.

4-(1*H*-indol-5-yl)isoquinoline (22a). The title compound was synthesized according to Method A using crude 5-bromoindole (250 mg, 1.26 mmol), isoquinoline-4-ylboronic acid (240 mg, 1.39 mmol), sodium carbonate (667 mg, 6.30 mmol) and tetrakis(triphenylphosphine)palladium (0) (73 mg, 0.06 mmol) in dimethoxyethane (12 mL) and water (4 mL). The crude product was purified by flash column chromatography on silica gel (EtOAc/n-hexane, 1:100 to 1:2) to yield a yellow solid (190 mg, 62%).

4-(1-Acetyl-1*H*-indol-5-yl)isoquinoline (22). The title compound was synthesized according to Method D using **22a** (190 mg, 0.78 mmol), triethylamine (0.16 mL, 1.17 mmol), acetic anhydride (0.28 mL, 3.03 mmol) and DMAP (27 mg, 0.22 mmol) and obtained as a pale yellow solid (130 mg, 58%). mp 120–122 °C, R_f = 0.18 (EtOAc/n-hexane, 1:1). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 2.70 (s, 3H), 6.73 (d, J = 3.7 Hz, 1H), 7.51 (dd, J = 1.7, 8.5 Hz, 1H), 7.53 (d, J = 3.7 Hz, 1H), 7.66 (m, 2H), 7.70 (d, J = 1.4 Hz, 1H), 7.94 (d, J = 8.4 Hz, 1H), 8.06 (dd, J = 1.3, 7.4 Hz, 1H), 8.54 (s, 1H), 8.59 (d, J = 8.4 Hz, 1H), 9.28 (s, 1H). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 24.0, 109.2, 116.2, 122.3, 125.0, 126.0, 127.2, 127.3, 127.9, 128.4, 130.7, 130.8, 132.4, 133.6, 134.6, 135.2, 142.7, 151.6, 168.6. MS (ESI) m/z = 287 $[M+H]^+$.

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Supporting Information

1. The synthetic procedures and characterization of intermediates 1c, 1b, 10b and 14b as well as final compounds 3–9.

***tert*-Butyl 5-bromo-2,3-dihydro-1*H*-indole-1-carboxylate (1c).** To a solution of 5-bromoindoline (1.20 g, 6.10 mmol) in anhydrous THF (20 mL) and water (20 mL) was added $NaHCO_3$ (1.53g, 18.2 mmol). After being cooled down to 0 °C, to the reaction mixture was added Boc_2O (2.59 mL, 12.1 mmol) dropwise, and stirred at the same temperature for 30 min. Afterwards, the reaction was warmed to room temperature and stirred for additional 5 h. The resulting mixture was separated and the aqueous layer was extracted with EtOAc (3 x 20 mL), dried over $MgSO_4$ and concentrated in vacuo to give a grey solid (2.85 g), which was directly used in next step without further purification.

***tert*-Butyl 5-pyridin-3-yl-2,3-dihydro-1*H*-indole-1-carboxylate (1b).** The title compound were synthesized according to Method A using crude **1c** (2.85 g), pyridin-3-ylboronic acid (0.98 g, 7.93 mmol), sodium carbonate (3.23 g, 30.5 mmol) and tetrakis(triphenylphosphine)palladium (0) (0.35 g, 0.30 mmol) in dimethoxyethane (30 mL) and water (10 mL). The crude product was purified by flash column chromatography on silica gel (EtOAc/n-hexane, 1:100 to 1:2) to yield colorless crystals (1.42 g, 78% for two steps). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 1.58 (s, 9H), 3.16 (t, J = 8.7 Hz, 2H), 4.04 (t, J = 7.9 Hz, 2H), 7.32 (dd, J = 4.9, 7.9 Hz, 1H), 7.38 (m, 2H),7.74 (s, 1H), 7.83 (dt, J = 2.0, 7.9 Hz, 1H), 8.54 (dd, J = 1.5, 4.8 Hz, 1H), 8.81 (d, J = 2.2 Hz, 1H).

***tert*-Butyl 5-isoquinolin-4-yl-2,3-dihydro-1*H*-indole-1-carboxylate (10b).** The title compound were synthesized according to Method A using crude **10c** (1.61 g), isoquinoline-4-ylboronic acid (0.73 g, 4.24 mmol), sodium carbonate (1.87 g, 17.6 mmol) and tetrakis(triphenylphosphine)palladium (0) (0.20 g, 0.18 mmol) in dimethoxyethane (30 mL) and water (10 mL). The crude product was used directly to next step without further purification as brown solids (1.97 g).

1-Acetyl-5-bromo-2,3-dihydro-1*H*-indole (14b). The title compound were synthesized according to Method C using 5-bromoindoline (1.80 g, 9.09 mmol), acyl chloride (0.62 mL, 10.9 mmol), pyridine (1.10 mL, 13.6 mmol) and anhydrous THF (35 mL) to yield the crude product as grey solids (1.92 g) without further purification.

1-(3-Chloropropanoyl)-5-pyridin-3-yl-2,3-dihydro-1*H*-indole (3). The title compound was synthesized

according to Method C using **1a** (80 mg, 0.41 mmol), 3-chloropropanoyl chloride (48 µL, 0.49 mmol) and pyridine (50 µL, 0.62 mmol) in anhydrous THF (3 mL). After flash column chromatography, recrystallization from THF gave pale yellow needles (95 mg, 81%). mp 157–159 °C, R_f = 0.10 (EtOAc/n-hexane, 1:1). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 2.95 (t, *J* = 6.8 Hz, 2H), 3.30 (t, *J* = 8.5 Hz, 2H), 3.93 (t, *J* = 6.8 Hz, 2H), 4.15 (t, *J* = 8.5 Hz, 2H), 7.34 (dd, *J* = 4.8, 7.8 Hz, 1H), 7.44 (m, 2H), 7.84 (dt, *J* = 1.9, 7.8 Hz, 1H), 8.31 (d, *J* = 8.2 Hz, 1H), 8.56 (d, *J* = 4.5 Hz,1H), 8.82 (d, *J* = 1.9 Hz, 1H).^{13}C-NMR (125 MHz, $CDCl_3$): δ 28.0, 38.7, 39.2, 48.2, 117.5, 123.2, 123.5, 126.7, 132.2, 133.6, 134.0, 136.2, 142.7, 148.1, 148.2, 168.9. MS (ESI) *m/z* = 287 M^+.

1-(2-Methylpropanoyl)-5-pyridin-3-yl-2,3-dihydro-1*H*-indole (4). The title compound was synthesized according to Method C using **1a** (100 mg, 0.51 mmol), isobutyryl chloride (64 µL, 0.61 mmol) and pyridine (62 µL, 0.77 mmol) in anhydrous THF (5 mL). The crude product underwent flash column chromatography twice gave pale yellow crystals (90 mg, 66%). mp 135–137 °C, R_f = 0.08 (EtOAc/n-hexane, 1:1). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 1.25 (d, *J* = 6.7 Hz, 6H), 2.80 (m, 1H), 3.27 (t, *J* = 7.8 Hz, 2H), 4.19 (t, *J* = 8.5 Hz, 2H), 7.34 (dd, *J* = 4.9, 7.7 Hz, 1H), 7.42 (m, 2H), 7.85 (dt, *J* = 2.0, 7.9 Hz, 1H), 8.36 (d, *J* = 7.5 Hz, 1H), 8.55 (dd, *J* = 0.9, 4.5 Hz, 1H), 8.82 (d, *J* = 1.9 Hz, 1H). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 19.1, 28.0, 33.5, 48.1, 117.7, 123.0, 123.5, 126.6, 132.3, 132.3, 133.0, 134.1, 136.4, 143.5, 147.9, 175.8. MS (ESI) *m/z* = 267 $[M+H]^+$.

1-(Cyclopropylcarbonyl)-5-pyridin-3-yl-2,3-dihydro-1*H*-indole (5). The title compound was synthesized according to Method C using **1a** (100 mg, 0.51 mmol), cyclopropanecarbonyl chloride (56 µL, 0.61 mmol) and pyridine (62 µL, 0.77 mmol) in anhydrous THF (5 mL). The crude product underwent flash column chromatography twice gave pale yellow crystals (103 mg, 76%). mp 158–161 °C, R_f = 0.07 (EtOAc/n-hexane, 1:1). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 0.91 (m, 2H), 1.15 (m, 2H), 1.78 (s, br, 1H), 3.30 (t, *J* = 8.3 Hz, 2H), 4.33 (t, *J* = 8.3 Hz, 2H), 7.34 (dd, *J* = 4.8, 7.8 Hz, 1H), 7.40 (m, 2H), 7.85 (dt, *J* = 2.0, 7.8 Hz, 1H), 8.26 (s, br, 1H), 8.55 (d, *J* = 4.6 Hz,1H), 8.82 (d, *J* = 1.8 Hz, 1H).^{13}C-NMR (125 MHz, $CDCl_3$): δ 8.3, 13.6, 27.9, 48.3, 117.3, 123.1, 123.5, 126.6, 132.2, 132.8, 134.1, 136.4, 143.4, 147.8, 171.9. MS (ESI) *m/z* = 265 $[M+H]^+$.

1-[(4-Fluorophenyl)carbonyl]-5-pyridin-3-yl-2,3-dihydro-1*H*-indole (6). The title compound was synthesized according to Method C using **1a** (80 mg, 0.41 mmol), 4-fluorobenzoyl chloride (60 µL, 0.49 mmol) and pyridine (50 µL, 0.62 mmol) in anhydrous THF (3 mL). After flash column chromatography, recrystallization from THF gave pale yellow crystals (102 mg, 78%). mp 161–162 °C, R_f = 0.09 (EtOAc/n-hexane, 1:1). ^{1}H-NMR (500 MHz, DMSO-d_6, 300K): δ 3.16 (t, *J* = 8.3 Hz, 2H), 4.07 (t, *J* = 8.3 Hz, 2H), 7.34 (m, 2H), 7.46 (dd, *J* = 4.8, 8.0 Hz, 1H), 7.56 (s, br, 1H), 7.67 (d, *J* = 0.9 Hz,1H), 7.70 (m, 2H), 8.05 (d, *J* = 8.0 Hz, 1H), 8.53 (dd, *J* = 1.5, 4.7 Hz, 1H), 8.87 (d, *J* = 1.8 Hz,1H). ^{1}H-NMR (500 MHz, DMSO-d_6, 373K): δ 3.19 (t, *J* = 8.3 Hz, 2H), 4.08 (t, *J* = 8.3 Hz, 2H), 7.31 (m, 2H), 7.43 (dd, *J* = 4.7, 7.9 Hz, 1H), 7.51 (d, *J* = 8.3 Hz, 1H), 7.63 (s, 1H), 7.68 (m, 2H), 7.73 (s, br, 1H), 8.01 (dt, *J* = 1.9, 7.9 Hz, 1H), 8.53 (dd, *J* = 1.4, 4.7 Hz, 1H), 8.85 (d, *J* = 2.0 Hz, 1H). ^{13}C-NMR (125 MHz, DMSO-d_6): δ 27.7, 50.5, 115.5 (d, $^2J_{C,F}$ = 22.0 Hz), 116.8, 123.4, 123.8, 125.6, 129.7 (d, $^3J_{C,F}$ = 8.8 Hz), 132.5, 133.3, 133.6, 133.9, 135.2, 142.7, 147.3, 148.0,

163.0 (d, $^{1}J_{C,F}$ = 247 Hz), 167.2. MS (ESI) *m/z* = 319 $[M+H]^{+}$.

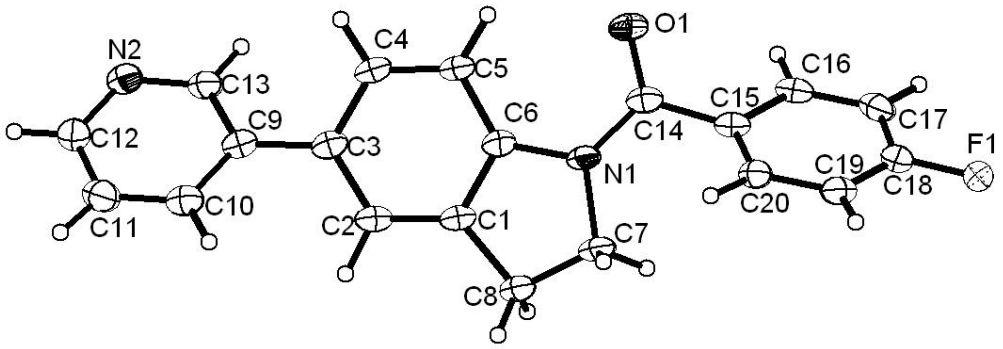
1-[(4-Methoxyphenyl)carbonyl]-5-pyridin-3-yl-2,3-dihydro-1*H*-indole (7). The title compound was synthesized according to Method C using **1a** (80 mg, 0.41 mmol), 4-methoxybenzoyl chloride (70 µL, 0.49 mmol) and pyridine (50 µL, 0.62 mmol) in anhydrous THF (3 mL). After flash column chromatography ($MeOH/CH_2Cl_2$, 0 to 1:100), recrystallization from THF gave colorless crystals (106 mg, 78%). mp 177–179 °C, R_f = 0.07 (EtOAc/n-hexane, 1:1). ^{1}H-NMR (500 MHz, DMSO-d_6, 300K): δ 3.16 (t, *J* = 8.3 Hz, 2H), 3.83 (s, 3H), 4.11 (t, *J* = 8.3 Hz, 2H), 7.04 (m, 2H), 7.45 (dd, *J* = 4.8, 7.9 Hz, 1H), 7.54 (d, *J* = 7.7 Hz, 1H), 7.60 (m, 2H), 7.66 (s, 1H), 8.04 (dt, *J* = 2.0, 8.0 Hz, 1H), 8.52 (dd, *J* = 1.5, 4.7 Hz, 1H), 8.87 (d, *J* = 2.0 Hz, 1H). ^{1}H-NMR (500 MHz, DMSO-d_6, 373K): δ 3.18 (t, *J* = 8.3 Hz, 2H), 3.86 (s, 3H), 4.12 (t, *J* = 8.3 Hz, 2H), 7.04 (m, 2H), 7.42 (dd, *J* = 4.7, 7.9 Hz, 1H), 7.47 (dd, *J* = 1.7, 8.3 Hz, 1H), 7.57 (m, 2H), 7.60 (d, *J* = 0.9 Hz, 1H), 7.67 (d, *J* = 8.4 Hz, 1H), 8.98 (dt, *J* = 2.0, 7.9 Hz, 1H), 8.52 (dd, *J* = 1.5, 4.7 Hz, 1H), 8.84 (d, *J* = 2.4 Hz, 1H).^{13}C-NMR (125 MHz, $CDCl_3$): δ 28.2, 50.9, 55.4, 113.8, 117.2, 123.5, 126.3, 128.8, 129.4, 132.1, 133.3, 133.5, 133.9, 136.3, 143.1, 148.1, 148.2, 161.5, 168.9. MS (ESI) *m/z* = 331 $[M+H]^{+}$.

5-Pyridin-3-yl-1-(thiophen-2-ylcarbonyl)-2,3-dihydro-1*H*-indole (8). The title compound was synthesized according to Method C using **1a** (80 mg, 0.41 mmol), thiophene-2-carbonyl chloride (50 µL, 0.49 mmol) and pyridine (50 µL, 0.62 mmol) in anhydrous THF (3 mL). Purification by flash column chromatography ($MeOH/CH_2Cl_2$, 0 to 1:100) gave pale yellow solids (101 mg, 80%). mp 160–162 °C, R_f = 0.08 (EtOAc/n-hexane, 1:1). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 3.30 (t, *J* = 8.3 Hz, 2H), 4.45 (t, *J* = 8.3 Hz, 2H), 7.13 (t, *J* = 8.3 Hz, 1H), 7.34 (dd, *J* = 4.8, 7.9 Hz, 1H), 7.44 (m, 2H), 7.56 (dd, *J* = 0.9, 5.0 Hz, 1H), 7.62 (dd, *J* = 0.8, 3.7 Hz, 1H), 7.83 (dt, *J* = 2.0, 7.9 Hz, 1H), 8.18 (s, br, 1H), 8.56 (dd, *J* = 1.5, 4.8 Hz, 1H), 8.83 (d, *J* = 2.0 Hz, 1H).^{13}C-NMR (125 MHz, $CDCl_3$): δ 28.7, 50.8, 118.2, 123.2, 123.5, 126.6, 127.3, 130.0, 130.4, 132.9, 133.8, 134.0, 136.2, 139.2, 143.3, 148.1, 148.2, 161.5. MS (ESI) *m/z* = 307 $[M+H]^{+}$.

1-(Penylacetyl)-5-pyridin-3-yl-2,3-dihydro-1*H*-indole (9). The title compound was synthesized according to Method C using **1a** (80 mg, 0.41 mmol), phenylacetyl chloride (70 µL, 0.49 mmol) and pyridine (50 µL, 0.62 mmol) in anhydrous THF (3 mL). After flash column chromatography ($MeOH/CH_2Cl_2$, 0 to 1:100), recrystallization from THF gave pale yellow crystals (110 mg, 85%). mp 125–127 °C, R_f = 0.08 (EtOAc/n-hexane, 1:1). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 3.24 (t, *J* = 8.5 Hz, 2H), 3.84 (s, 2H), 4.13 (t, *J* = 8.5 Hz, 2H), 7.28 (m, 1H), 7.34 (m, 5H), 7.39 (s, 1H), 7.42 (d, *J* = 8.5 Hz,1H), 7.83 (dt, *J* = 1.8, 7.9 Hz, 1H), 8.36 (d, *J* = 8.4 Hz, 1H), 8.55 (d, *J* = 1.3, 4.7 Hz, 1H), 8.81 (d, *J* = 2.1 Hz, 1H).^{13}C-NMR (125 MHz, $CDCl_3$): δ 28.0, 43.5, 48.4, 117.6, 123.1, 123.5, 126.7, 127.1, 128.8, 129.0, 132.2, 133.4, 134.0, 134.0, 136.3, 143.2, 148.0, 148.1, 169.2. MS (ESI) *m/z* = 315 $[M+H]^{+}$.

2. X-ray crystal structure data of compound 6 and 7.

Compound **6**:



Crystal data and structure refinement.

Empirical formula	$C_{20}H_{15}FN_2O$	
Formula weight	318.34	
Temperature	153(2) K	
Wavelength	0.71073 Å	
Crystal system	Triclinic	
Space group	P-1	
Unit cell dimensions	a = 9.3874(5) Å	α = 101.696(3)°.
	b = 11.5729(6) Å	β = 102.001(3)°.
	c = 16.2624(11) Å	γ = 111.851(2)°.
Volume	1525.71(15) $Å^3$	
Z	4	
Density (calculated)	1.386 mg/m^3	
Absorption coefficient	0.095 mm^{-1}	
F(000)	664	
Crystal size	0.43 x 0.38 x 0.25 mm^3	
Theta range for data collection	1.35 to 27.37°.	
Index ranges	-12<=h<=12, -14<=k<=14, -19<=l<=20	
Reflections collected	24262	
Independent reflections	6722 [R(int) = 0.0407]	
Completeness to theta = 27.37°	97.4 %	
Absorption correction	Multiscan	
Max. and min. transmission	0.9768 and 0.9600	

Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	6722 / 0 / 553
Goodness-of-fit on F^2	1.067
Final R indices [I>2sigma(I)]	R1 = 0.0505, wR2 = 0.1410
R indices (all data)	R1 = 0.0687, wR2 = 0.1547
Largest diff. peak and hole	0.332 and -0.276 e.Å^{-3}

Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters (Å^2 x 10^3) for Compound **6**. U(eq) is defined as one third of the trace of the orthogonalized U^{ij} tensor.

	x	y	z	U(eq)
N(1)	4623(2)	6365(1)	1256(1)	27(1)
N(2)	6959(2)	488(2)	933(1)	35(1)
O(1)	1972(2)	5173(1)	1066(1)	37(1)
F(1)	1461(2)	10378(1)	948(1)	44(1)
C(1)	6629(2)	5696(2)	1180(1)	26(1)
C(2)	7356(2)	4860(2)	1197(1)	29(1)
C(3)	6564(2)	3647(2)	1326(1)	27(1)
C(4)	5040(2)	3331(2)	1443(1)	30(1)
C(5)	4300(2)	4162(2)	1436(1)	30(1)
C(6)	5109(2)	5352(2)	1297(1)	26(1)
C(7)	5994(3)	7516(2)	1214(2)	38(1)
C(8)	7195(2)	7000(2)	1016(2)	33(1)
C(9)	7307(2)	2728(2)	1340(1)	28(1)
C(10)	8952(2)	3148(2)	1755(1)	32(1)
C(11)	9571(3)	2242(2)	1763(1)	36(1)
C(12)	8537(3)	927(2)	1355(2)	35(1)
C(13)	6382(2)	1381(2)	931(1)	30(1)
C(14)	3077(2)	6224(2)	1132(1)	27(1)
C(15)	2732(2)	7371(2)	1090(1)	26(1)
C(16)	1287(2)	7138(2)	478(1)	29(1)
C(17)	848(2)	8136(2)	425(1)	31(1)
C(18)	1867(2)	9384(2)	1001(1)	31(1)
C(19)	3264(2)	9647(2)	1631(1)	30(1)
C(20)	3706(2)	8641(2)	1673(1)	28(1)
N(3)	3263(2)	5050(2)	3730(1)	26(1)
N(4)	6053(2)	-466(2)	4145(1)	37(1)
O(2)	975(2)	4146(1)	4091(1)	35(1)
F(2)	157(2)	9095(2)	3526(1)	60(1)

C(21)	5390(2)	4486(2)	3809(1)	25(1)
C(22)	6131(2)	3664(2)	3813(1)	26(1)
C(23)	5251(2)	2355(2)	3762(1)	25(1)
C(24)	3615(2)	1931(2)	3712(1)	28(1)
C(25)	2853(2)	2750(2)	3709(1)	28(1)
C(26)	3763(2)	4043(2)	3755(1)	25(1)
C(27)	4599(2)	6193(2)	3651(2)	33(1)
C(28)	6085(2)	5919(2)	3874(1)	30(1)
C(29)	6001(2)	1435(2)	3757(1)	25(1)
C(30)	7244(2)	1514(2)	3395(1)	29(1)
C(31)	7867(2)	610(2)	3413(1)	32(1)
C(32)	7237(3)	-364(2)	3788(1)	34(1)
C(33)	5470(2)	420(2)	4126(1)	31(1)
C(34)	1881(2)	5042(2)	3884(1)	26(1)
C(35)	1474(2)	6149(2)	3780(1)	27(1)
C(36)	805(2)	6622(2)	4378(1)	30(1)
C(37)	365(2)	7618(2)	4301(2)	35(1)
C(38)	578(2)	8106(2)	3605(2)	38(1)
C(39)	1161(2)	7628(2)	2982(2)	38(1)
C(40)	1631(2)	6651(2)	3076(1)	31(1)

Bond lengths [Å] and angles [°] for Compound **6**.

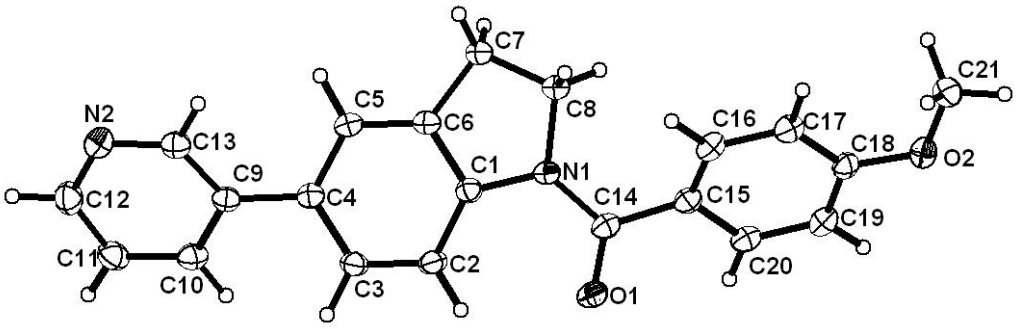
N(1)-C(14)	1.364(2)
N(1)-C(6)	1.416(2)
N(1)-C(7)	1.495(2)
N(2)-C(13)	1.333(3)
N(2)-C(12)	1.342(3)
O(1)-C(14)	1.236(2)
F(1)-C(18)	1.353(2)
C(1)-C(2)	1.377(3)
C(1)-C(6)	1.396(3)
C(1)-C(8)	1.502(3)
C(2)-C(3)	1.404(3)
C(3)-C(4)	1.403(3)
C(3)-C(9)	1.474(3)
C(4)-C(5)	1.381(3)
C(5)-C(6)	1.394(2)
C(7)-C(8)	1.519(3)

C(9)-C(10)	1.397(3)
C(9)-C(13)	1.400(3)
C(10)-C(11)	1.375(3)
C(11)-C(12)	1.386(3)
C(14)-C(15)	1.491(3)
C(15)-C(16)	1.400(3)
C(15)-C(20)	1.403(3)
C(16)-C(17)	1.376(3)
C(17)-C(18)	1.389(3)
C(18)-C(19)	1.372(3)
C(19)-C(20)	1.383(3)
N(3)-C(34)	1.369(2)
N(3)-C(26)	1.414(2)
N(3)-C(27)	1.497(2)
N(4)-C(33)	1.332(3)
N(4)-C(32)	1.335(3)
O(2)-C(34)	1.231(2)
F(2)-C(38)	1.363(2)
C(21)-C(22)	1.372(3)
C(21)-C(26)	1.395(2)
C(21)-C(28)	1.510(2)
C(22)-C(23)	1.405(2)
C(23)-C(24)	1.406(3)
C(23)-C(29)	1.478(3)
C(24)-C(25)	1.384(3)
C(25)-C(26)	1.397(2)
C(27)-C(28)	1.532(3)
C(29)-C(30)	1.394(3)
C(29)-C(33)	1.402(3)
C(30)-C(31)	1.377(3)
C(31)-C(32)	1.387(3)
C(34)-C(35)	1.496(3)
C(35)-C(36)	1.391(3)
C(35)-C(40)	1.394(3)
C(36)-C(37)	1.381(3)
C(37)-C(38)	1.381(3)
C(38)-C(39)	1.364(3)
C(39)-C(40)	1.382(3)
C(14)-N(1)-C(6)	124.57(15)
C(14)-N(1)-C(7)	125.66(16)

C(6)-N(1)-C(7)	109.00(15)
C(13)-N(2)-C(12)	116.98(18)
C(2)-C(1)-C(6)	120.45(16)
C(2)-C(1)-C(8)	129.73(17)
C(6)-C(1)-C(8)	109.78(17)
C(1)-C(2)-C(3)	120.02(17)
C(4)-C(3)-C(2)	118.19(18)
C(4)-C(3)-C(9)	120.56(16)
C(2)-C(3)-C(9)	121.25(17)
C(5)-C(4)-C(3)	122.55(17)
C(4)-C(5)-C(6)	117.87(18)
C(5)-C(6)-C(1)	120.90(18)
C(5)-C(6)-N(1)	129.16(17)
C(1)-C(6)-N(1)	109.94(15)
N(1)-C(7)-C(8)	104.94(16)
C(1)-C(8)-C(7)	104.40(16)
C(10)-C(9)-C(13)	116.68(19)
C(10)-C(9)-C(3)	122.29(17)
C(13)-C(9)-C(3)	121.03(17)
C(11)-C(10)-C(9)	119.69(19)
C(10)-C(11)-C(12)	118.8(2)
N(2)-C(12)-C(11)	123.3(2)
N(2)-C(13)-C(9)	124.52(19)
O(1)-C(14)-N(1)	121.32(18)
O(1)-C(14)-C(15)	119.80(17)
N(1)-C(14)-C(15)	118.87(15)
C(16)-C(15)-C(20)	118.67(18)
C(16)-C(15)-C(14)	117.52(17)
C(20)-C(15)-C(14)	123.60(17)
C(17)-C(16)-C(15)	121.03(18)
C(16)-C(17)-C(18)	118.38(18)
F(1)-C(18)-C(19)	118.55(18)
F(1)-C(18)-C(17)	118.98(18)
C(19)-C(18)-C(17)	122.47(19)
C(18)-C(19)-C(20)	118.77(18)
C(19)-C(20)-C(15)	120.62(18)
C(34)-N(3)-C(26)	124.77(15)
C(34)-N(3)-C(27)	125.69(16)
C(26)-N(3)-C(27)	108.89(15)
C(33)-N(4)-C(32)	116.96(17)

C(22)-C(21)-C(26)	120.94(16)
C(22)-C(21)-C(28)	129.56(17)
C(26)-C(21)-C(28)	109.48(16)
C(21)-C(22)-C(23)	120.07(17)
C(22)-C(23)-C(24)	118.01(17)
C(22)-C(23)-C(29)	121.73(16)
C(24)-C(23)-C(29)	120.26(16)
C(25)-C(24)-C(23)	122.54(17)
C(24)-C(25)-C(26)	117.86(17)
C(21)-C(26)-C(25)	120.58(17)
C(21)-C(26)-N(3)	110.51(15)
C(25)-C(26)-N(3)	128.88(17)
N(3)-C(27)-C(28)	104.69(15)
C(21)-C(28)-C(27)	103.94(15)
C(30)-C(29)-C(33)	116.46(18)
C(30)-C(29)-C(23)	123.27(16)
C(33)-C(29)-C(23)	120.27(17)
C(31)-C(30)-C(29)	119.49(17)
C(30)-C(31)-C(32)	119.14(19)
N(4)-C(32)-C(31)	123.1(2)
N(4)-C(33)-C(29)	124.83(19)
O(2)-C(34)-N(3)	121.54(18)
O(2)-C(34)-C(35)	120.30(17)
N(3)-C(34)-C(35)	118.14(15)
C(36)-C(35)-C(40)	119.28(19)
C(36)-C(35)-C(34)	117.87(17)
C(40)-C(35)-C(34)	122.68(17)
C(37)-C(36)-C(35)	120.55(19)
C(36)-C(37)-C(38)	118.1(2)
F(2)-C(38)-C(39)	118.3(2)
F(2)-C(38)-C(37)	118.5(2)
C(39)-C(38)-C(37)	123.1(2)
C(38)-C(39)-C(40)	118.3(2)
C(39)-C(40)-C(35)	120.6(2)

Compound **7**:



Crystal data and structure refinement.		
Empirical formula	$C_{21}H_{18}N_2O_2$	
Formula weight	330.37	
Temperature	153(2) K	
Wavelength	0.71073 Å	
Crystal system	Monoclinic	
Space group	C2/c	
Unit cell dimensions	a = 19.5025(4) Å	α = 90°.
	b = 11.0639(2) Å	β = 109.506(2)°.
	c = 15.5364(4) Å	γ = 90°.
Volume	3159.94(12) $Å^3$	
Z	8	
Density (calculated)	1.389 mg/m^3	
Absorption coefficient	0.090 mm^{-1}	
F(000)	1392	
Crystal size	0.30 x 0.25 x 0.13 mm^3	
Theta range for data collection	2.15 to 27.90°.	
Index ranges	-18<=h<=25, -14<=k<=14, -20<=l<=20	
Reflections collected	15711	
Independent reflections	3782 [R(int) = 0.0356]	
Completeness to theta = 27.90°	99.8 %	

Absorption correction	Multiscan
Max. and min. transmission	0.9883 and 0.9731
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	3782 / 0 / 298
Goodness-of-fit on F^2	1.029
Final R indices [I>2sigma(I)]	R1 = 0.0377, wR2 = 0.0887
R indices (all data)	R1 = 0.0547, wR2 = 0.0973
Largest diff. peak and hole	0.255 and -0.200 e.Å^{-3}

Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters (Å^2 x 10^3) for Compound **7**. U(eq) is defined as one third of the trace of the orthogonalized U^{ij} tensor.

	x	y	z	U(eq)
N(1)	4633(1)	9286(1)	3532(1)	22(1)
N(2)	8676(1)	11194(1)	5779(1)	29(1)
O(1)	4446(1)	7294(1)	3703(1)	34(1)
O(2)	1150(1)	8388(1)	1616(1)	29(1)
C(1)	5388(1)	9273(1)	4038(1)	20(1)
C(2)	5856(1)	8296(1)	4337(1)	23(1)
C(3)	6576(1)	8530(1)	4856(1)	22(1)
C(4)	6842(1)	9706(1)	5066(1)	20(1)
C(5)	6360(1)	10674(1)	4748(1)	21(1)
C(6)	5640(1)	10454(1)	4246(1)	20(1)
C(7)	5028(1)	11342(1)	3895(1)	23(1)
C(8)	4386(1)	10561(1)	3327(1)	25(1)
C(9)	7619(1)	9933(1)	5585(1)	21(1)
C(10)	8033(1)	9186(1)	6292(1)	25(1)
C(11)	8758(1)	9456(1)	6732(1)	29(1)
C(12)	9055(1)	10457(1)	6455(1)	31(1)
C(13)	7977(1)	10918(1)	5368(1)	24(1)
C(14)	4194(1)	8286(1)	3401(1)	23(1)
C(15)	3397(1)	8411(1)	2902(1)	22(1)
C(16)	3085(1)	9146(1)	2147(1)	24(1)
C(17)	2338(1)	9163(1)	1699(1)	25(1)
C(18)	1887(1)	8434(1)	2005(1)	23(1)
C(19)	2194(1)	7672(1)	2753(1)	25(1)
C(20)	2936(1)	7656(1)	3184(1)	24(1)
C(21)	819(1)	9208(1)	877(1)	33(1)

Bond lengths [Å] and angles [°] for Compound **7**.

N(1)-C(14)	1.3726(15)
N(1)-C(1)	1.4184(15)
N(1)-C(8)	1.4907(15)
N(2)-C(13)	1.3343(16)
N(2)-C(12)	1.3394(18)
O(1)-C(14)	1.2295(15)
O(2)-C(18)	1.3614(15)
O(2)-C(21)	1.4360(17)
C(1)-C(2)	1.3906(17)
C(1)-C(6)	1.3953(16)
C(2)-C(3)	1.3905(18)
C(3)-C(4)	1.3986(17)
C(4)-C(5)	1.4022(17)
C(4)-C(9)	1.4808(17)
C(5)-C(6)	1.3796(17)
C(6)-C(7)	1.5018(17)
C(7)-C(8)	1.5337(18)
C(9)-C(13)	1.3944(17)
C(9)-C(10)	1.3952(17)
C(10)-C(11)	1.3817(18)
C(11)-C(12)	1.384(2)
C(14)-C(15)	1.4939(17)
C(15)-C(16)	1.3890(17)
C(15)-C(20)	1.3999(17)
C(16)-C(17)	1.3885(18)
C(17)-C(18)	1.3895(18)
C(18)-C(19)	1.3981(18)
C(19)-C(20)	1.3773(18)
C(14)-N(1)-C(1)	123.70(10)
C(14)-N(1)-C(8)	126.22(10)
C(1)-N(1)-C(8)	109.19(9)
C(13)-N(2)-C(12)	116.34(12)
C(18)-O(2)-C(21)	117.26(10)
C(2)-C(1)-C(6)	120.55(11)
C(2)-C(1)-N(1)	129.54(11)
C(6)-C(1)-N(1)	109.89(10)
C(3)-C(2)-C(1)	118.17(11)

C(2)-C(3)-C(4)	122.24(11)
C(3)-C(4)-C(5)	118.30(11)
C(3)-C(4)-C(9)	121.16(11)
C(5)-C(4)-C(9)	120.51(11)
C(6)-C(5)-C(4)	120.05(11)
C(5)-C(6)-C(1)	120.67(11)
C(5)-C(6)-C(7)	128.68(11)
C(1)-C(6)-C(7)	110.58(11)
C(6)-C(7)-C(8)	103.75(10)
N(1)-C(8)-C(7)	105.50(10)
C(13)-C(9)-C(10)	116.65(11)
C(13)-C(9)-C(4)	120.23(11)
C(10)-C(9)-C(4)	123.11(11)
C(11)-C(10)-C(9)	119.30(12)
C(10)-C(11)-C(12)	118.92(12)
N(2)-C(12)-C(11)	123.57(13)
N(2)-C(13)-C(9)	125.22(12)
O(1)-C(14)-N(1)	120.81(11)
O(1)-C(14)-C(15)	119.73(11)
N(1)-C(14)-C(15)	119.45(11)
C(16)-C(15)-C(20)	117.90(11)
C(16)-C(15)-C(14)	125.38(11)
C(20)-C(15)-C(14)	116.54(11)
C(17)-C(16)-C(15)	121.43(12)
C(16)-C(17)-C(18)	119.88(12)
O(2)-C(18)-C(17)	124.38(11)
O(2)-C(18)-C(19)	116.24(11)
C(17)-C(18)-C(19)	119.38(12)
C(20)-C(19)-C(18)	120.02(12)
C(19)-C(20)-C(15)	121.34(12)

Acknowledgements. The authors appreciate Dr. Volker Huch for the determination and analysis of X-ray crystal structure.

3. HPLC purity control of all final compounds.

The Surveyor®-LC-system consisted of a pump, an autosampler, and a PDA detector. Mass spectrometry was performed on a TSQ® Quantum (Thermo Electron Corporation, Dreieich, Germany). The triple quadrupole mass spectrometer was equipped with an electrospray interface (ESI). The system was operated by the standard software Xcalibur®.

A RP C18 NUCLEODUR® 100-5 (125 × 3 mm) column (Macherey-Nagel GmbH, Duehren, Germany) was used as stationary phase. All solvents were HPLC grade.

In a gradient run the percentage of acetonitrile (containing 0.1% triflouro-acetic acid) in water was increased from an initial concentration of 3% at 0 min to 100% at 15 min and kept at 100% for 3 min.

The injection volume was 10 µl and flow rate was set to 350 µl/min. MS analysis was carried out at a spray voltage of 3800 V, a capillary temperature of 350 °C and a source CID of 10 V. Spectra were acquired in positive mode from 100 to 1000 m/z and full scan UV-mode. In some cases APC ionization had to be applied.

Compd.	RT (min)	Purity [%]
1	9.08	99.7%
2	7.79	99.9%
3	10.86	99.6%
4	8.93	99.9%
5	8.64	99.9%
6	10.33	99.9%
7	9.92	99.9%
8	9.71	99.9%
9	10.44	99.9%
10	9.03	99.7%
11	12.26	99.7%
12	10.36	99.7%
13	10.61	99.9%
14	11.17	99.9%
15	11.24	99.9%
16	8.35	99.4%
17	9.70	99.9%
18	7.60	99.9%
19	11.05	99.7%
20	7.43	99.9%
21	8.16	98.5%
22	10.09	99.7%

3.III. Novel Pyridinylmethyl Substituted 1,2,5,6-Tetrahydro-pyrrolo[3,2,1-*ij*]quinolin-4-ones Type Dual CYP19 / CYP11B2 Inhibitors as Adjuvant Therapy for Post-Menopausal Breast Cancer Patients with Elevated Risk for Cardiovascular Complications

Introduction

Estrogens not only act physiologically as important sexual hormones to promote the development of female secondary sexual characteristics and to maintain the normal functions of the reproductive system, but also exhibit dichotomous impacts on human health under some pathological circumstances. On one hand, estrogens stimulate the proliferation of breast cancer (BC) cells which express estrogen receptors (ER) and / or progesterone receptors (PgR).[1] Estrogen deprivation is therefore a rational treatment for BC. Two approaches have been implemented in clinic: selective estrogen receptor modulators (SERMs),[2] and aromatase inhibitors (AIs),[3] such as letrozole and vorozole (Chart 1). Since AIs show fewer side effects compared to SERMs especially in long-term applications, they are first choice nowadays as adjuvant therapeutics for postmenopausal women, the majority of breast cancer patients. On the other hand, estrogen deficiency is closely correlated with cardiovascular diseases (CVD), which has been demonstrated by the fact that the incidences of CVD in post-menopausal women triple those of premenopausal women at the same age.[4] It has been elucidated that estrogens exhibit some protective effects on heart[5] and kidney.[6] The administration of estrogen prevents the development of heart failure post-myocardial infarction[7] and attenuates ventricular hypertrophy and remodelling.[8] As for BC patients, menopause and the application of AIs decrease the estrogen concentrations below undetectable levels,[9] which leads to even higher risk of CVD.

It is believed that the high CVD risk results from estrogen depletion and is mediated to a great extent by the increase of aldosterone levels. Estrogen deficiency not only directly promotes aldosterone secretion, but also upregulates other renin–angiotensin–aldosterone system (RAAS) components, such as rennin, angiotensin II (Ang II), angiotensin converting enzyme (ACE), and angiotensin II type 1 receptor (AT_1R), which further elevate aldosterone biosynthesis.[10] The abnormally high concentration of aldosterone retains sodium and water leading to an increase of blood volume and the subsequent elevation of blood pressure.[11] It

also promotes the influx of calcium into vascular smooth muscle cells[12] and the expression of adrenomedullin and regulator of G protein signaling-2 (RGS2)[13] leading to vasoconstriction, which together with the increase of blood volume results in chronic hypertension. Moreover, excessive aldosterone acts as a pro-inflammation factor[14], and it induces the production of reactive oxygen species (ROS)[15] as well. Inflammation and ROS formation lead to cardiac myocyte necrosis, collagen synthesis and fibroblast proliferation thus resulting in cardiac and vascular fibrosis and an increase in myocardial stiffness.[16] Subsequently, cardiac hypertrophy and ventricular remodelling occure as further structural deterioration with functional degradation.[17] The ventricular remodeling causes diastolic dysfunction, diminishes contractile capability, reduces stroke volume and ultimately results in heart failure, often leading to sudden death.

Aldosterone synthase (CYP11B2) is the crucial enzyme catalyzing the conversion of 11-deoxycorticosterone to aldosterone. Its inhibition leading to a reduction of aldosterone levels would be beneficial for post-menopausal BC patients under AI treatment. For pursuing this goal, the dual inhibitors of CYP19 and CYP11B2 would be advantageous for reasons of a better compliance and to decrease side effects. Besides, application of this kind of a dual-target-directed agents[18] could also avoid possible drug-drug interactions often observed after of administration of two drugs. Accordingly, in this paper we like to develop dual inhibitors of both enzymes as a novel innovative therapeutic approach for the treatment of BC.

For this, important structural features of CYP11B2 inhibitors and CYP19 inhibitors were combined in a common structural template and a series of pyridinylmethyl substituted 1,2,5,6-tetrahydro-pyrrolo[3,2,1-*ij*]quinolin-4-ones **1**–**26** were synthesized. The inhibition of CYP11B2 and CYP19 by these compounds is presented in comparison with fadrozole (Chart 1), which is a potent CYP19 inhibitor showing unselective inhibition of 11β-hydroxylase (CYP11B1) and CYP11B2. Accordingly, the selectivity of the new compounds against CYP11B1 and 17α-hydroxylase-17,20-lyase (CYP17), which are the crucial enzymes in the biosyntheses of glucocorticoids and androgens, respectively, were also determined as safety criteria.

Dual Inhibitors Design Concept

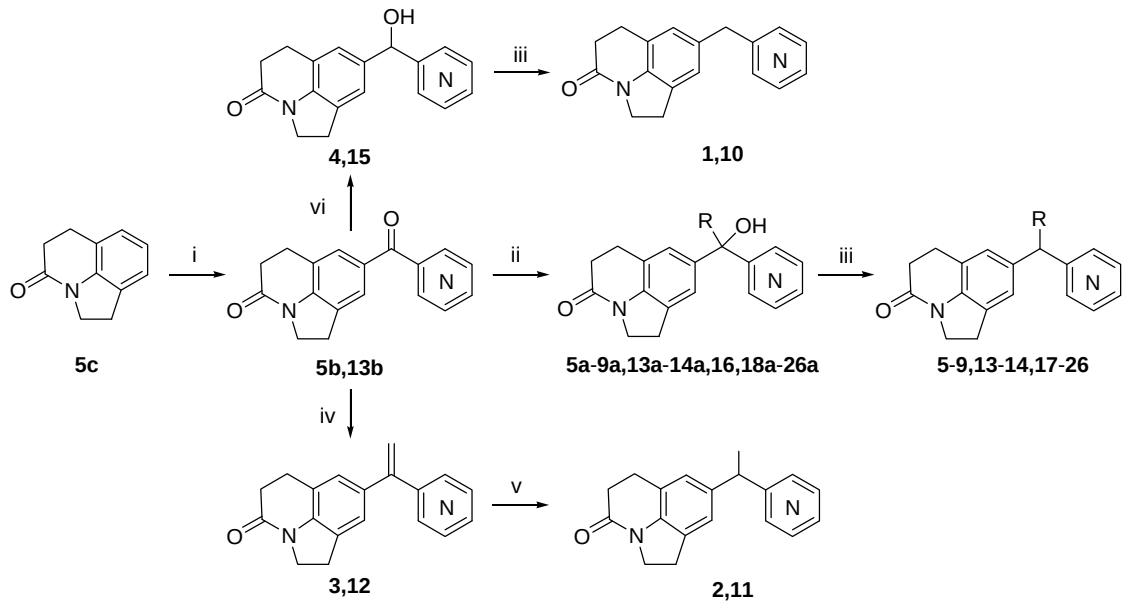
All cytochrome P450s are cysteinato-heme enzymes, and the iron in the protoporphyrin acts as the reactive centre to activate oxygen which is required for the oxidation of the substrate. Therefore, reversible competitive inhibition via the coordination of this Fe by sp^2 hybrid N is a common inhibitory mechanism for most of the CYP enzymes inhibitors. It was first identified for CYP19 inhibitors,[19a] but was soon successfully applied to the inhibitors of CYP17,[20] CYP11B1,[21] and CYP11B2.[22] Despite poor sequence identity of less than 20% across the whole CYP superfamily, CYP proteins share similar folding configurations and conserved substrate binding pockets. This makes it difficult to achieve selectivity, but could be advantageous to develop dual inhibitors. A simple design strategy was applied in this study: the combination of the important structural features of selective CYP19 and CYP11B2 inhibitors into one molecule. Plenty of CYP19 inhibitors[19] have been designed and synthesized with letrozole and vorozole (Chart 1) as the representatives. It is obvious that three major structural features appear in these two compounds: triazole providing sp^2 hybrid N, CH between N containing heterocycle and hydrophobic core, and two hydrophobic aryls. These two compounds showed potent inhibition of CYP19 and weak or no

intereference with CYP11B2. On the contrary, fadrozole (Chart 1), in which one aryl was replaced by alkyl fused onto the imidazole, was not selective toward CYP11B1 and CYP11B2 inhibition. Hence, the scaffold of arylmethyl substituted N containing heterocycle was considered to be crucial for strong CYP19 inhibition and modifications on aryl and N containing heterocycle were performed to tune the inhibition of CYP11B enzymes. As our efforts to develop selective CYP11B2 inhibitors had been successful and had led to reference compound **I**[22h] with 1,2,5,6-tetrahydro-pyrrolo[3,2,1-*ij*]quinolin-4-one as hydrophobic core and 3-pyridyl as N containing heterocycle. This core was considered to be important for a strong CYP11B2 inhibition. It was therefore introduced into the CYP19 scaffold to achieve dual inhibition. Moreover, 3- or 4-pyridinyl were employed as N-containing heterocycles while various substituents were furnished on the CH bridge leading to a series of pyridinylmethyl substituted 1,2,5,6-tetrahydro-pyrrolo[3,2,1-*ij*]quinolin-4-ones (Chart 1).

Results and Discussion

Chemistry. The preparation of compounds **1**–**26** is illustrated in Scheme 1. The 1,2,5,6-tetrahydro-pyrrolo[3,2,1-*ij*]quinolin-4-one core **5c** was synthesized from indoline after amidation and Friedel-Crafts alkylation, and subsequently converted into the pyridinyl ketones **5b** (3-py) and **13b** (4-py) via Friedel-Crafts acylation with the corresponding nicotinoyl or isonicotinoyl chlorides in solid phase. The ketones were transformed into alcohols **5a**–**9a**, **13a**–**15a, 17a**–**26a** and **16** with various Grignard reagents, whose OH

Scheme 1[a]



	Het.	R		Het.	R		Het.	R
1	3-Py	-	**10**	4-Py	-	**19**	4-Py	3-MeO Ph
2	3-Py	-	**11**	4-Py	-	**20**	4-Py	4-MeO Ph
3	3-Py	-	**12**	4-Py	-	**21**	4-Py	3-F Ph
4	3-Py	-	**13**	4-Py	*i*-Pr	**22**	4-Py	4-F Ph
5	3-Py	Ph	**14**	4-Py	*c*-Hex	**23**	4-Py	3-Cl Ph
6	3-Py	2-MeO Ph	**15**	4-Py	OH	**24**	4-Py	4-Cl Ph
7	3-Py	3-Me Ph	**16**	4-Py	Ph, OH	**25**	4-Py	3-Me Ph
8	3-Py	3-Cl Ph	**17**	4-Py	Ph	**26**	4-Py	3,5-di CF_3 Ph
9	3-Py	4-F Ph	**18**	4-Py	2-MeO Ph			

[a] Reagents and conditions: (i) Method A: Nicotinoyl or isonicotinoyl chloride hydrochloride, $AlCl_3$, 140 °C; (ii) Method B: RMgX, THF, –78 °C to room temp.; (iii) Method C: Et_3SiH, TFA, CF_3SO_3H, CH_2Cl_2, 0 °C to room temp.; (iv) Method D: CH_3PPh_3Br, n-BuLi, THF, –78 °C to room temp.; (v) Method E: Pd/C, H_2, MeOH, room temp.; (vi) Method F: $NaBH_4$, MeOH, 0 °C.

groups were then removed using triethylsilane under acidic conditions to afford compounds **5**–**9**, **13**–**15**, **17**–**26** with various substituents at the CH bridge. On the contrary, reduction of ketones by sodium borohydride followed by triethylsilane treatment led to non-substituted analogues **1** and **10**. Moreover, methylene group was introduced onto the CH bridge via Wittig reaction (compounds **3** and **12**), which were further saturated by hydrogenation to yield **2** and **11.**Our group has reported about several series of biphenyl methylene imidazoles[13] as potent CYP17 inhibitors. All these compounds were designed based on the mechanism that the sp^2 hybrid nitrogen can coordinate with the heme iron, which was first[14a] identified for aromatase (CYP19, estrogen synthase) inhibitors,[14] and later was also proven to be valid for aldosterone synthase (CYP11B2)[15] and CYP17 inhibitors.[10, 12-13]

Biology.

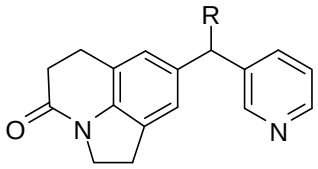
Inhibition of Human CYP19, CYP11B2 and CYP11B1. The synthesized compounds were investigated for their inhibitory activities against CYP19 with human placenta microsomal preparations[23a] as well as against CYP11B1 and CYP11B2 in V79 MZh cells expressing the corresponding enzymes.[23b,c] IC_{50} values are presented in comparison to lead compounds fadrozole and letrozol.

It is obvious that when 3-pyridinyl was employed as heterocycle to provide the sp^2 hybrid N, the resulting compounds **1**–**8** showed weak to no inhibition of CYP19 regardless of the substituents on the CH bridge (Table 1). Exception was compound **9** with 4-F Ph at the CH bridge exhibiting potent inhibition with an IC_{50} value of 140 nM. On the contrary, some compounds were much more potent toward CYP11B2. Analogues with no (**1**) or methyl (**2**) substituents on the CH bridge were strong CYP11B2 inhibitors with IC_{50} values of 74 and 37 nM, respectively. Introduction of an unsaturated methylene group (**3**) decreased the inhibitory activity to 468 nM, probably due to the rigidification limiting the flexibility. The total loss of potency rendered by OH (**4**) leads to the conclusion that hydrophilicity is not tolerated in this area. Moreover, strict steric limitation was observed for compounds **5**–**9**. When substituted with a phenyl (**5**), the compound turned out to be potent (IC_{50} = 132 nM). However, further introduction of methoxy (**6**), methyl (**7**) and chloro (**8**) substituents into the phenyl at the 2- or 3- positions led to total loss of inhibitory potency (IC_{50} > 5000 nM). Although small groups like 4-F (**9**) also reduced the potency, it was less pronounced (IC_{50} = 587 nM). After complexing the metal ion by the perpendicular interaction between N and Fe, it is difficult for the substituted phenyl groups to fit into the hydrophobic pocket.

Interestingly, all the 3-pyridyl analogues showed preference for CYP11B1 with selectivity factors (SF = $IC_{50\,CYP11B1}$ / $IC_{50\,CYP11B2}$) less than 0.1.

As for 4-pyridyl analogues, much more potent inhibitors of CYP19 and CYP11B2 were obtained (Table 2). When alkyl groups were introduced to the CH bridge, completely opposite SARs were observed for the inhibition of CYP19 or CYP11B2. As the bulkiness of the substituents increased from H (**10**) to methyl (**11**), isopropyl (**13**) and cyclohexyl (**14**), the inhibitory potency toward CYP19 increased from 2130 nM, 1500 nM, 889 nM to 74 nM, respectively. However, this enhancement of bulkiness resulted in the reduction of CYP11B2 inhibition from 204 nM (**10**, H) to 1290 nM (**14**, cyclohexyl). Among these compounds, methyl

Table 1. Inhibition of CYP19, CYP11B1, CYP11B2, and CYP17 by 3-pyridinyl compounds **1**–**9**.



1-9

Compd	R	IC_{50}[a] nM CYP19[c]	IC_{50}[a] nM		SF[f]	Inhib.%[b] CYP17[g]
			CYP11B2[d]	CYP11B1[e]		
1	H	3340	74	16	0.2	0.5
2	Me	> 5000	37	4	0.1	0.4
3	$=CH_2$	> 5000	468	41	0.1	3.1
4	OH	> 5000	> 5000	750	< 0.1	n.d.[i]
5	Ph	2130	132	68	0.1	n.d.[i]
6	2-MeO Ph	3660	> 5000	2270	< 0.3	n.d.[i]
7	3-Me Ph	> 5000	> 5000	1220	< 0.2	n.d.[i]
8	3-Cl Ph	1350	> 5000	36	< 0.1	n.d.[i]
9	4-F Ph	140	587	78	0.1	n.d.[i]
I		18%[h]	1.1	715	650	6
Fadrozole		41	0.8	6.3	7.9	0
Letrozole		36	1420	2620	1.8	6.8

[a] Mean value of at least three experiments, relative standard deviation usually less than 25%. [b] Mean value of at least two experiments, standard deviation usually less than 10%; inhibitor concentration, 500 nM. [c] Human placental CYP19; substrate androstenedione, 500 nM, inhibitor concentration 500 nM; fadrozole, IC_{50} = 41 nM; [d] Hamster fibroblasts expressing human CYP11B2; substrate deoxycorticosterone, 100 nM. [e] Hamster fibroblasts expressing human CYP11B1; substrate deoxycorticosterone, 100 nM. [f] $SF = IC_{50\ CYP11B1} / IC_{50\ CYP11B2}$. [g] *E. coli* expressinghuman CYP17; substrate progesterone, 25 μM; inhibitor concentration 2.0 μM; ketoconazole, IC_{50} = 3.5 μM. [h] Inhibition percentage at inhibitor concentration of 500 nM. [i] n.d.: not determined.

derivative **11** as an exception increased in the potency (IC_{50} = 74 nM), probably caused by the hydrophobic interaction between methyl and the small pocket which it fits in. Although potent inhibitors for CYP19 or CYP11B2 were identified respectively, no compromise of the contradictory SARs can be achieved to afford potent dual inhibitors. Moreover, these compounds exhibited no or little selectivity with SF ranging from 1 to 4. Nevertheless, compound **12** with methylene at the CH bridge showed good selectivity over CYP11B1 (SF = 13). This compounds also exhibited potent inhibition of CYP11B2 (IC_{50} = 96 nM) and modest inhibition of CYP19 (IC_{50} = 565 nM). Similar as observed for 3-pyridinyl analogues, OH (**15** and **17**) significantly decreased the inhibitory potency of both enzymes.

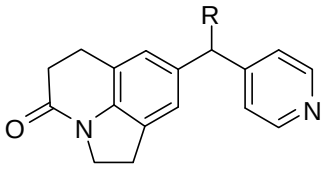
Furthermore, bulky phenyl substitution on the CH bridge led to potent dual inhibitors. Consistent with the SAR observed above for CYP19, the increase in bulkiness leading to substituted phenyl resulted in strong inhibition of CYP19 with IC_{50} values ranging from 19 nM to 124 nM. Interestingly, the phenyl group also rendered potent CYP11B2 inhibition with IC_{50} values between 32 nM and 139 nM. This is in contrast to the low inhibition of analogues with other bulky groups, such as isopropyl (**13**, IC_{50} = 750 nM) and cyclohexyl

(**14**, IC_{50} = 1290 nM). This intriguing finding implies that the hydrophobic pocket in CYP11B2 might be narrow and rather flat so that the isopropyl or cyclohexyl group cannot fit in well. Possible π-π interactions between phenyl and some amino acid residues in the pocket might be responsible for this difference as well. Contrary influences on the inhibition of CYP19 and CYP11B2 were observed for the H-bond acceptors (OMe and F) on the phenyl ring. Regarding CYP19, the 2- and 3-methoxy substituted compounds **18** and **19** showed elevated inhibition compared to non-substituted **16** (IC_{50} = 105 nM) with IC_{50} values of 81 and 59 nM, respectively, whereas the 4-MeO analogue **20** exhibited slightly decreased inhibitory activity (IC_{50} = 124 nM). On the contrary, as for CYP11B2 inhibition, the 2- or 3-MeO Ph substitution led to slight reduction of CYP11B2 inhibition (IC_{50} values around 130 nM) compared to **16** (IC_{50} = 85 nM), while the 4-MeO Ph compound **20** are more potent (IC_{50} = 52 nM). Similar observations were also made for F analogues **21** and **22**: when the compound showed improved inhibition toward one enzyme, the inhibitory potency toward the other enzyme was reduced and vice versa. Nonetheless, the substitution of chloro (**23** and **24**) and methyl (**25**) on the phenyl moiety significantly increased the inhibitory potency toward both enzymes regardless of the substitution positions leading to potent dual inhibitors of CYP19 and CYP11B2 (IC_{50} values less than 50 nM for both enzymes). Although **24** with 4-Cl Ph substitution exhibited poor selectivity (SF = 2.9) between CYP11B2 and CYP11B1, the other two compounds **23** (3-Cl Ph) and **25** (3-CH_3 Ph) showed good selectivity with SFs of 13 and 33, respectively. Especially, compound **25** appeared to be superior to the lead compounds fadrozle and letrozole when taking into consideration the inhibitory potency towards both CYP19 and CYP11B2 as well as the selectivity between CYP11B2 and CYP11B1. It is interesting to observe that compounds with 3-substituted Ph moiety are always more selective than the corresponding 4-substituted Ph analogues (**19** & **20**, **21** & **22** and **23** & **24**). Also, 3-substituted Ph compounds are more potent toward CYP19, whereas 4-substituted Ph derivatives inhibit CYP11B2 in a strong way. Furthermore, the total loss of CYP11B2 inhibition for compound **26**, substituted with two trifluoromethyl groups at the *m*-positions, is a further indication of the tightness of the pocket.

The potent ($IC_{50\ CYP19}$ = 124 nM and $IC_{50\ CYP11B2}$ = 52 nM) and selective (regarding CYP11B1, SF = 16) dual inhibitor **20** was resolved into pure enantiomers to examine the influence of the chiral center on biological activity. One enantiomer **20-1** showed enhanced CYP11B2 inhibition (IC_{50} = 46 nM) and selectivity (SF = 37), however, the CYP19 inhibitory activity was reduced (IC_{50} = 273 nM). Contrarily, the other enantiomer **20-2** exhibited stronger inhibition of CYP19 (IC_{50} = 92 nM), and CYP11B2 inhibition (IC_{50} = 74 nM) and selectivity (SF = 7) were decreased. Since it is the aim to achieve potent dual inhibition, the racemate is considered to optimal.

Inhibition of Human CYP17. The inhibition of CYP17 by the synthesized compounds was evaluated due to its important role in the biosynthesis of androgens. The percent inhibition values were obtained using the 50,000 g sediment of *E.coli* coexpressing human CYP17 and cytochrome P450 reductase at an inhibitor concentration of 2 μM.[23d,e] The potent dual inhibitors **16**, **18**–**25** showed weak inhibition (around 50 % at 2 μM) toward CYP17, which is tolerable regarding their potency against CYP19 and CYP11B2 (IC_{50} values ranging from 19 to 139 nM).

Table 2. Inhibition of CYP19, CYP11B1, CYP11B2, and CYP17 by 4-pyridinyl compounds **10**–**26**.



10-26

Compd	R	IC_{50}[a] nM CYP19[c]	IC_{50}[a] nM		SF[f]	Inhib.%[b] CYP17[g]
			CYP11B2[d]	CYP11B1[e]		
10	H	2130	204	224	1.1	8.2
11	Me	1500	74	140	1.9	n.d.[i]
12	$=CH_2$	565	96	1230	13	17
13	*i*-Pr	889	750	1420	1.9	n.d.[i]
14	*c*-Hex	74	1290	> 5000	> 4	n.d.[i]
15	OH	> 5000	563	347	1.6	0.6
16	Ph	105	85	745	8.8	43
17	Ph, OH	880	390	5399	14	n.d.[i]
18	2-MeO Ph	81	128	276	2.2	33
19	3-MeO Ph	59	139	2539	18	50
20	4-MeO Ph	124	52	810	16	53
20-1	4-MeO Ph	273	46	1688	37	n.d.[i]
20-2	4-MeO Ph	92	74	511	6.9	n.d.[i]
21	3-F Ph	74	88	532	6.0	n.d.[i]
22	4-F Ph	116	56	88	1.6	55
23	3-Cl Ph	19	51	646	13	58
24	4-Cl Ph	55	32	92	2.9	60
25	3-CH_3 Ph	32	41	1336	33	46
26	3,5-diCF_3 Ph	246	1580	> 5000	> 3	n.d.[i]
I		18%[h]	1.1	715	650	6
Fadrozole		41	0.8	6.3	7.9	0
Letrozole		36	1420	2620	1.8	6.8

[a] Mean value of at least three experiments, relative standard deviation usually less than 25%. [b] Mean value of at least two experiments, standard deviation usually less than 10%; inhibitor concentration, 500 nM. [c] Human placental CYP19; substrate androstenedione, 500 nM, inhibitor concentration 500 nM; fadrozole, IC_{50} = 41 nM; [d] Hamster fibroblasts expressing human CYP11B2; substrate deoxycorticosterone, 100 nM. [e] Hamster fibroblasts expressing human CYP11B1; substrate deoxycorticosterone, 100 nM. [f] $SF = IC_{50\ CYP11B1} / IC_{50\ CYP11B2}$. [g] *E. coli* expressinghuman CYP17; substrate progesterone, 25 μM; inhibitor concentration 2.0 μM; ketoconazole, IC_{50} = 3.5 μM. [h] Inhibition percentage at inhibitor concentration of 500 nM. [i] n.d.: not determined.

Conclusion

Estrogen deficiency has been observed to be closely correlated with CVD, which is believed to be mediated to a large extent by abnormally high concentration of aldosterone. The application of AIs to post-

menopausal BC patients reduces estrogens to undetectable levels leading to high risk of CVD. Since AIs as adjuvant therapy have to be applied for more than five years, it is rational to design dual inhibitors of CYP19 and CYP11B2 to reduce CVD risk.

By integrating the 1,2,5,6-tetrahydro-pyrrolo[3,2,1-*ij*]quinolin-4-one core from CYP11B2 inhibitors into the CYP19 inhibitor template of arylmethyl substituted N containing heterocycles, a series of potent dual inhibitors were designed and synthesized. It has been found that 3-pyridinyl was not suitable for this purpose, whereas 4-pyridinyl led to potent dual inhibitors. The substituents on the CH bridge showed significant influence on the inhibitory activity, in which opposite SARs were commonly observed for the inhibition of these two enzymes. When alkyl groups were furnished, the enhancement of bulkiness increased CYP19 inhibition, but reduced that of CYP11B2. When substituted phenyl groups were introduced, the resulting compounds exhibited strong inhibition toward both enzymes. Depending on the substitution position, H-bond acceptors, such as methoxy and fluoro at the phenyl moiety, resulted in different inhibitory potencies. Opposite SARs were observed: the shifting of methoxy or fluoro from 2- or 3-position to 4-position decreased CYP19 inhibition, but elevated potency toward CYP11B2. Contrarily, chloro and methyl substitution led to potent dual inhibitors of CYP19 and CYP11B2 regardless their positions on the phenyl ring. It is interesting that 3-substituted Ph compounds are more potent toward CYP19, whereas 4-substituted Ph derivatives show a stronger inhibition of CYP11B2, and compounds with 3-substitution Ph are more selective than the corresponding 4-substituted Ph analogues. Moreover, the resolution of a racemic mixture led to an increase in CYP19 inhibition but to a reduction of CYP11B2 inhibition and selectivity regarding CYP11B1 for one enantiomer; while for the other one opposite results were observed. The compromise of these conflicts led to compounds **19**, **20**, **23** and **25** as potent and selective dual inhibitors of CYP19 and CYP11B2, especially compound **25**, which exhibited IC_{50} values of 32 and 41 nM for CYP19 and CYP11B2, respectively, and a SF of 33 over CYP11B1. This compound is deemed to be superior to the leads fadrozole and letrozole, and could be a drug candidate after further evaluation in vivo.

Experimental Section

Biology

CYP19 preparation and assay

Human CYP19 was obtained from microsomal preparations of human placenta and the assay was performed using the ^3H_2O-method as previously described and [1β-^{3}H] androstenedione as substrate.[23a]

Inhibition of CYP11B1 and CYP11B2

V79MZh cells expressing human CYP11B1 or CYP11B2 were incubated with [1,2-^{3}H]-11-deoxycorticosterone as substrate and the inhibitor at different concentrations. The assay was performed as previously described.[23b,c]

CYP17 preparation and assay

Human CYP17 was expressed in *E. coli*[23d] (coexpressing human CYP17 and NADPH-P450 reductase),

and the assay was performed using method as previously described, progesterone as substrate and NADPH as cofactor.[23e]

Chemistry

General Method

Melting points were determined on a Mettler FP1 melting point apparatus and are uncorrected. ^{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. All coupling constants (*J*) are given in Hz. ESI (electrospray ionization) mass spectra were determined on a TSQ quantum (Thermo Electron Corporation) instrument. The purities of the final compounds were controlled by Surveyor-LC-system. Purities were greater than 95%. 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). Reagents and solvents were used as obtained from commercial suppliers without further purification.

Resolution of Racemate. Enantiomers were separated via preparative HPLC on a Agilent Technologies 1200 series system (quaternary pump, MWD, fraction collector) using a NucleoCel Delta column, 0.8 cm × 25 cm. Retention times (t_R) were determined with an analytical column using 0.3 mL/min and UV detection (254 nm). The ee values were measured on a Merck-Hitachi LaChrome D-7000 System (isocratic pump L7100, diode array detector L7455, autosampler L-7200) using a Chiracel OD-H column, 0.46 cm × 25 cm.

Method A: Friedel-Crafts Acylation

1,2,5,6-Tetrahydro-4*H*-pyrrolo[3,2,1-*ij*]quinolin-4-one (1.0 equiv), nicotinoyl chloride hydrochloride or isonicotinoyl chloride hydrochloride (1.5 equiv) and $AlCl_3$ (5.0 equiv) were melted at 140 °C for 4 h followed by being cooled to 0 °C. A mixture of ice/water was added to decompose the excessive $AlCl_3$, and the resulting mixture was stirred at ambient temperature for 1 h. Extraction with $CHCl_3$ (3 x 20 mL) gave the organic layers, which were combined and dried over $MgSO_4$. After removal of solvent in vacuo, the residue was purified by flash chromatography on silica gel to yield the corresponding ketones.

8-(Pyridin-3-ylcarbonyl)-1,2,5,6-tetrahydro-4*H*-pyrrolo[3,2,1-*ij*]quinolin-4-one (5b). The title compound was obtained according to Method A using 1,2,5,6-tetrahydro-4*H*-pyrrolo[3,2,1-*ij*]quinolin-4-one (0.50 g, 2.89 mmol), nicotinoyl chloride hydrochloride (0.77 g, 4.33 mmol) and $AlCl_3$ (2.60 g,19.5 mmol). The crude product was purified by flash chromatography column on silica gel (methanol/dichloromethane, 1/100, R_f = 0.07) to give yellow solids (0.69 g, 86%), mp 135–137 °C. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 2.72 (t, *J* = 7.8 Hz, 2H), 3.03 (t, *J* = 7.8 Hz, 2H), 3.25 (t, *J* = 8.5 Hz, 2H), 4.16 (t, *J* = 8.5 Hz, 2H), 7.45 (ddd, *J* = 0.8, 4.9, 7.9 Hz, 1H), 7.54 (s, 1H), 7.59 (s, 1H), 8.08 (dt, *J* = 2.0, 7.9 Hz, 1H), 8.80 (dd, *J* = 1.7, 4.7 Hz, 1H), 8.94 (dd, *J* = 0.8, 2.2 Hz, 1H). MS (ESI) *m/z* = 279 $[M+H]^+$.

8-(Pyridin-4-ylcarbonyl)-1,2,5,6-tetrahydro-4*H*-pyrrolo[3,2,1-*ij*]quinolin-4-one (13b). The title compound was obtained according to Method A using 1,2,5,6-tetrahydro-4*H*-pyrrolo[3,2,1-*ij*]quinolin-4-one (**5c**, 1.00 g, 5.77 mmol), isonicotinoyl chloride hydrochloride (1.54 g, 8.65 mmol) and $AlCl_3$ (3.85 g, 28.9 mmol). The crude product was purified by flash chromatography column on silica gel

(methanol/dichloromethane, 1/100, R_f = 0.05) to give yellow solids (1.15 g, 72%), mp 171–173 °C. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 2.74 (t, *J* = 7.8 Hz, 2H), 3.03 (t, *J* = 7.9 Hz, 2H), 3.24 (t, *J* = 8.5 Hz, 2H), 4.16 (t, *J* = 8.5 Hz, 2H), 7.52 (dd, *J* = 1.6, 4.4 Hz, 2H), 7.53 (s, 1H), 7.58 (s, 1H), 8.80 (dd, *J* = 1.6, 4.4 Hz, 2H). MS (ESI) *m/z* = 279 $[M+H]^+$.

Method B: Grignard Addition

To a solution of a ketone (1.0 equiv) in anhydrous THF was added dropwise a solution of an appropriate Grignard reagent (2.0–3.0 equiv) under an atmosphere of nitrogene at –78 °C. The reaction was stirred at the same temperature for another 1h, and subsequently warmed to room temperature. After being stirred for 15 h, the reaction was quenched with saturated aqueous $NHCl_4$ (5 mL), extracted with ethyl acetate (3 x 10 mL), dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by flash chromatograph on silica gel (methanol/dichloromethane, 0 to 1:30) to yield the corresponding alcohol.

Method C: Triethylsilane Reduction

To an alcohol (1.0 equiv) in anhydrous dichloromethane was added in sequence by syringe trifluoroacetic acid (10 equiv), triethylsilane (3.0 equiv) and trifluoromethanesulfuric acid (0.1 equivalent) under an atmosphere of nitrogen at 0 °C. The resulting solution was stirred at room temperature for 18–48 h. Afterwards, the reaction mixture was separated, and the queous layer was extracted with dichloromethane (2 x 10 mL). The combined organic layers were washed with aqueous $NaHCO_3$ and brine, dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by flash chromatograph on silica gel (methanol/dichloromethane, 0 to 1:40) to yield the corresponding dehydoxyl product.

8-(Pyridin-3-ylmethyl)-1,2,5,6-tetrahydro-4*H*-pyrrolo[3,2,1-*ij*]quinolin-4-one (1). The title compound was obtained according to Method C using **4** (70 mg, 0.25 mmol), trifluoroacetic acid (0.19 mL, 2.50 mmol), triethylsilane (0.12 mL, 0.75 mmol) and trifluoromethanesulfuric acid (2 µL, 0.02 mmol) in anhydrous CH_2Cl_2 (5 mL) as white solids (44 mg, 67%), mp 108–110 °C. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 2.65 (t, *J* = 7.7 Hz, 2H), 2.92 (t, *J* = 7.7 Hz, 2H), 3.14 (t, *J* = 8.5 Hz, 2H), 3.90 (s, 2H), 4.06 (t, *J* = 8.5 Hz, 2H), 6.80 (s, 1H), 6.88 (s, 1H), 7.20 (dd, *J* = 4.8, 7.7 Hz, 1H), 8.46 (d, *J* = 7.8 Hz, 1H), 8.46 (dd, *J* = 1.1, 7.8 Hz, 1H), 8.49 (d, *J* = 1.3 Hz, 1H). MS (ESI) *m/z* = 265 $[M+H]^+$.

8-[Phenyl(pyridin-3-yl)methyl]-1,2,5,6-tetrahydro-4*H*-pyrrolo[3,2,1-*ij*]quinolin-4-one (5). The title compound was obtained according to Method C using **5a** (60 mg, 0.17 mmol), trifluoroacetic acid (0.13 mL, 1.68 mmol), triethylsilane (82 µL, 0.51 mmol) and trifluoromethanesulfuric acid (2 µL, 0.02 mmol) in anhydrous CH_2Cl_2 (3 mL) as pale yellow solids (46 mg, 81%), mp 142–144 °C. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 2.65 (t, *J* = 7.7 Hz, 2H), 2.89 (t, *J* = 7.7 Hz, 2H), 3.12 (t, *J* = 8.4 Hz, 2H), 4.06 (t, *J* = 8.4 Hz, 2H), 5.49 (s, 1H), 6.74 (s, 1H), 6.80 (s, 1H), 7.10 (m, 2H), 7.22 (dd, *J* = 4.7, 7.9 Hz, 1H), 7.24 (m, 1H), 7.31 (m, 2H), 7.40 (dt, *J* = 1.7, 7.9 Hz, 1H), 8.42 (d, *J* = 2.0 Hz, 1H), 8.48 (dd, *J* = 1.4, 4.7 Hz, 1H). MS (ESI) *m/z* = 341 $[M+H]^+$.

8-[(2-Methoxyphenyl)(pyridin-3-yl)methyl]-1,2,5,6-tetrahydro-4*H*-pyrrolo[3,2,1-*ij*]quinolin-4-one (6). The title compound was obtained according to Method C using **6a** (57 mg, 0.15 mmol), trifluoroacetic acid

(0.11 mL, 1.48 mmol), triethylsilane (71 µL, 0.44 mmol) and trifluoromethanesulfuric acid (2 µL, 0.02 mmol) in anhydrous CH_2Cl_2 (4 mL) as pale yellow solids (31 mg, 56%), mp 65–68 °C. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 2.65 (t, *J* = 7.7 Hz, 2H), 2.89 (t, *J* = 7.7 Hz, 2H), 3.12 (t, *J* = 8.5 Hz, 2H), 3.74 (s, 3H), 4.06 (t, *J* = 8.5 Hz, 2H), 5.85 (s, 1H), 6.71 (s, 1H), 6.78 (s, 1H), 6.84–6.91 (m, 3H), 7.20 (dd, *J* = 4.8, 7.8 Hz, 1H), 7.25 (m, 1H), 7.36 (dt, *J* = 1.7, 7.8 Hz, 1H), 8.38 (d, *J* = 2.1 Hz, 1H), 8.45 (dd, *J* = 1.5, 4.8 Hz, 1H). MS (ESI) *m/z* = 371 $[M+H]^+$.

8-[(3-Methylphenyl)(pyridin-3-yl)methyl]-1,2,5,6-tetrahydro-4*H*-pyrrolo[3,2,1-*ij*]quinolin-4-one (7). The title compound was obtained according to Method C using **7a** (62 mg, 0.17 mmol), trifluoroacetic acid (0.13 mL, 1.67 mmol), triethylsilane (0.81 µL, 0.50 mmol) and trifluoromethanesulfuric acid (2 µL, 0.02 mmol) in anhydrous CH_2Cl_2 (4 mL) as pale yellow solids (30 mg, 50%), mp 164–166 °C. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 2.31 (s, 3H), 2.65 (t, *J* = 7.7 Hz, 2H), 2.90 (t, *J* = 7.7 Hz, 2H), 3.12 (t, *J* = 8.4 Hz, 2H), 4.06 (t, *J* = 8.4 Hz, 2H), 5.44 (s, 1H), 6.73 (s, 1H), 6.80 (s, 1H), 6.88 (d, *J* = 7.6 Hz, 1H), 6.92 (s, 1H), 7.06 (d, *J* = 7.5 Hz, 1H), 7.18–7.23 (m, 2H), 7.40 (dd, *J* = 1.8, 7.9 Hz, 1H), 8.41 (d, *J* = 1.6 Hz, 1H), 8.47 (dd, *J* = 1.2, 4.7 Hz, 1H). MS (ESI) *m/z* = 355 $[M+H]^+$.

8-[(3-Chlorophenyl)(pyridin-4-yl)methyl]-1,2,5,6-tetrahydro-4*H*-pyrrolo[3,2,1-*ij*]quinolin-4-one (8). The title compound was obtained according to Method C using **8a** (72 mg, 0.18 mmol), trifluoroacetic acid (0.14 mL, 1.84 mmol), triethylsilane (0.89 µL, 0.55 mmol) and trifluoromethanesulfuric acid (2 µL, 0.02 mmol) in anhydrous CH_2Cl_2 (4 mL) as to give pale yellow solids (36 mg, 53 %), mp 129–131 °C. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 2.68 (t, *J* = 7.7 Hz, 2H), 2.93 (t, *J* = 7.7 Hz, 2H), 3.16 (t, *J* = 8.4 Hz, 2H), 4.10 (t, *J* = 8.4 Hz, 2H), 5.48 (s, 1H), 6.73 (s, 1H), 6.80 (s, 1H), 7.01 (m, 1H), 7.10 (s, 1H), 7.27 (m, 3H), 7.41 (d, *J* = 7.9 Hz, 1H), 8.43 (d, *J* = 1.9 Hz, 1H), 8.52 (dd, *J* = 1.3, 4.7 Hz, 1H). MS (ESI) *m/z* = 375 $[M+H]^+$.

8-[(4-Fluorophenyl)(pyridin-3-yl)methyl]-1,2,5,6-tetrahydro-4*H*-pyrrolo[3,2,1-*ij*]quinolin-4-one (9). The title compound was obtained according to Method C using **9a** (90 mg, 0.24 mmol), trifluoroacetic acid (0.18 mL, 2.40 mmol), triethylsilane (0.12 mL, 0.72 mmol) and trifluoromethanesulfuric acid (2 µL, 0.02 mmol) in anhydrous CH_2Cl_2 (5 mL) as pale yellow solids (50 mg, 58%), mp 175–177 °C. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 2.66 (t, *J* = 7.7 Hz, 2H), 2.90 (t, *J* = 7.7 Hz, 2H), 3.13 (t, *J* = 8.4 Hz, 2H), 4.07 (t, *J* = 8.4 Hz, 2H), 5.47 (s, 1H), 6.71 (s, 1H), 6.78 (s, 1H), 7.00 (m, 2H), 7.05 (m, 2H), 7.24 (dd, *J* = 4.8, 7.9 Hz, 1H), 7.38 (dt, *J* = 1.7, 7.9 Hz, 1H), 8.40 (d, *J* = 1.0 Hz, 1H), 8.49 (dd, *J* = 1.4, 4.7 Hz, 1H). MS (ESI) *m/z* = 359 $[M+H]^+$.

8-(Pyridin-4-ylmethyl)-1,2,5,6-tetrahydro-4*H*-pyrrolo[3,2,1-*ij*]quinolin-4-one (10). The title compound was obtained according to Method C using **15** (125 mg, 0.45 mmol), trifluoroacetic acid (0.34 mL, 4.46 mmol), triethylsilane (0.22 mL, 1.34 mmol) and trifluoromethanesulfuric acid (4 µL, 0.04 mmol) in anhydrous CH_2Cl_2 (5 mL) as pale yellow solids (65 mg, 55%), mp 199–200 °C. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 2.66 (t, *J* = 7.7 Hz, 2H), 2.93 (t, *J* = 7.7 Hz, 2H), 3.15 (t, *J* = 8.5 Hz, 2H), 3.89 (s, 2H), 4.07 (t, *J* = 8.5 Hz, 2H), 6.80 (s, 1H), 6.88 (s, 1H), 7.09 (dd, *J* = 1.4, 4.5 Hz, 2H), 8.50 (dd, *J* =1.4, 4.5 Hz, 2H). MS (ESI) *m/z* = 265 $[M+H]^+$.

8-(2-Methyl-1-pyridin-4-ylpropyl)-1,2,5,6-tetrahydro-4*H*-pyrrolo[3,2,1-*ij*]quinolin-4-one (13). The

title compound was obtained according to Method C using **13a** (167 mg, 0.52 mmol), trifluoroacetic acid (0.40 mL, 5.18 mmol), triethylsilane (0.25 mL, 1.55 mmol) and trifluoromethanesulfuric acid (5 µL, 0.05 mmol) in anhydrous CH_2Cl_2 (8 mL) as white solids (105 mg, 66 %), mp 137–139 °C. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 0.88 (q, J = 6.5 Hz, 6H), 2.40–2.47 (m, 1H), 2.64 (t, J = 7.7 Hz, 2H), 2.92 (t, J = 7.7 Hz, 2H), 3.14 (t, J = 8.4 Hz, 2H), 3.32 (d, J = 10.9 Hz, 1H), 4.04 (t, J = 8.4 Hz, 2H), 6.86 (s, 1H), 6.96 (s, 1H), 7.18 (dd, J = 1.4, 4.6 Hz, 2H), 8.47 (dd, J = 1.4, 4.6 Hz, 2H). MS (ESI) *m/z* = 307 $[M+H]^+$.

8-[Cyclohexyl(pyridin-3-yl)methyl]-1,2,5,6-tetrahydro-4*H*-pyrrolo[3,2,1-*ij*]quinolin-4-one (14). The title compound was obtained according to Method C using **14a** (150 mg, 0.41 mmol), trifluoroacetic acid (0.32 mL, 4.14 mmol), triethylsilane (0.20 mL, 1.24 mmol) and trifluoromethanesulfuric acid (4 µL, 0.04 mmol) in anhydrous CH_2Cl_2 (8 mL) as white crystals (80 mg, 54%), mp 83–85 °C. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 0.86 (m, 2H), 1.14–1.26 (m, 3H), 1.62–1.70 (m, 5H), 2.05 (m, 1H), 2.64 (t, J = 7.7 Hz, 2H), 2.92 (t, J = 7.7 Hz, 2H), 3.14 (t, J = 8.4 Hz, 2H), 3.40 (d, J = 10.9 Hz, 1H), 4.04 (t, J = 8.4 Hz, 2H), 6.85 (s, 1H), 6.95 (s, 1H), 7.17 (dd, J = 1.4, 4.6 Hz, 2H), 8.46 (dd, J = 1.4, 4.6 Hz, 2H). MS (ESI) *m/z* = 347 $[M+H]^+$.

8-[Phenyl(pyridin-4-yl)methyl]-1,2,5,6-tetrahydro-4*H*-pyrrolo[3,2,1-*ij*]quinolin-4-one (17). The title compound was obtained according to Method C using **17a** (70 mg, 0.20 mmol), trifluoroacetic acid (0.15 mL, 1.96 mmol), triethylsilane (95 µL, 0.59 mmol) and trifluoromethanesulfuric acid (2 µL, 0.02 mmol) in anhydrous CH_2Cl_2 (5 mL) as pale yellow solids (48 mg, 72%), mp 168–169 °C. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 2.66 (t, J = 7.7 Hz, 2H), 2.90 (t, J = 7.7 Hz, 2H), 3.13 (t, J = 8.4 Hz, 2H), 4.07 (t, J = 8.4 Hz, 2H), 5.43 (s, 1H), 6.73 (s, 1H), 6.79 (s, 1H), 7.04 (d, J = 5.3 Hz, 2H), 7.08 (d, J = 7.2 Hz, 2H), 7.26 (m, 1H), 7.32 (t, J = 7.4 Hz, 2H), 8.52 (d, J = 5.3 Hz, 2H). MS (ESI) *m/z* = 341 $[M+H]^+$.

8-[(2-Methoxyphenyl)(pyridin-4-yl)methyl]-1,2,5,6-tetrahydro-4*H*-pyrrolo[3,2,1-*ij*]quinolin-4-one (18). The title compound was obtained according to Method C using **18a** (77 mg, 0.20 mmol), trifluoroacetic acid (0.15 mL, 1.99 mmol), triethylsilane (97 µL, 0.60 mmol) and trifluoromethanesulfuric acid (2 µL, 0.02 mmol) in anhydrous CH_2Cl_2 (4 mL) as pale yellow solids (55 mg, 75%), mp 70–72 °C. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 2.66 (t, J = 7.7 Hz, 2H), 2.90 (t, J = 7.7 Hz, 2H), 3.12 (t, J = 8.5 Hz, 2H), 3.73 (s, 3H), 4.07 (t, J = 8.5 Hz, 2H), 5.80 (s, 1H), 6.71 (s, 1H), 6.78 (s, 1H), 6.83 (m, 1H), 6.90 (m, 2H), 7.00 (d, J = 4.6 Hz, 2H), 7.26 (m, 1H), 8.48 (d, J = 4.6 Hz, 2H). MS (ESI) *m/z* = 371 $[M+H]^+$.

8-[(3-Methoxyphenyl)(pyridin-4-yl)methyl]-1,2,5,6-tetrahydro-4*H*-pyrrolo[3,2,1-*ij*]quinolin-4-one (19). The title compound was obtained according to Method C using **19a** (102 mg, 0.26 mmol), trifluoroacetic acid (0.20 mL, 2.64 mmol), triethylsilane (0.13 mL, 0.79 mmol) and trifluoromethanesulfuric acid (2 µL, 0.03 mmol) in anhydrous CH_2Cl_2 (8 mL) as pale yellow solids (48 mg, 50%), mp 148–150 °C. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 2.66 (t, J = 7.7 Hz, 2H), 2.90 (t, J = 7.7 Hz, 2H), 3.12 (t, J = 8.4 Hz, 2H), 4.06 (t, J = 8.4 Hz, 2H), 5.39 (s, 1H), 6.63 (m, 1H), 6.68 (d, J = 7.7 Hz, 1H), 6.72 (s, 1H), 6.80 (m, 2H), 7.04 (d, J = 4.6 Hz, 2H), 7.24 (t, J = 8.0 Hz, 1H), 8.51 (d, J = 4.6 Hz, 2H). MS (ESI) *m/z* = 371 $[M+H]^+$.

8-[(4-Methoxyphenyl)(pyridin-4-yl)methyl]-1,2,5,6-tetrahydro-4*H*-pyrrolo[3,2,1-*ij*]quinolin-4-one (20). The title compound was obtained according to Method C using **20a** (102 mg, 0.26 mmol), trifluoroacetic acid (0.20 mL, 2.64 mmol), triethylsilane (0.13 mL, 0.79 mmol) and trifluoromethanesulfuric

acid (2 µL, 0.03 mmol) in anhydrous CH_2Cl_2 (8 mL) as pale yellow solids (83 mg, 86%), mp 71–73 °C. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 2.66 (t, *J* = 7.7 Hz, 2H), 2.90 (t, *J* = 7.7 Hz, 2H), 3.12 (t, *J* = 8.4 Hz, 2H), 3.80 (s, 3H), 4.06 (t, *J* = 8.4 Hz, 2H), 5.38 (s, 1H), 6.71 (s, 1H), 6.78 (s, 1H), 6.85 (d, *J* = 8.7 Hz, 2H), 7.00 (d, *J* = 8.7 Hz, 2H), 7.03 (d, *J* = 4.7 Hz, 2H), 8.51 (d, *J* = 4.7 Hz, 2H). MS (ESI) *m/z* = 371 $[M+H]^+$. The racemate **20** was separated by preparative HPLC on chiral stationary phase (NucleoCel Delta column, 0.8 cm × 25 cm, 50% hexane/isopropanol, 1.0 mL/min) to yield **20-1** (100% ee and t_R = 32.1 min by analytical HPLC) and **20-2** (90.3% ee and t_R = 38.2 min by analytical HPLC).

8-[(3-Fluorophenyl)(pyridin-4-yl)methyl]-1,2,5,6-tetrahydro-4*H*-pyrrolo[3,2,1-*ij*]quinolin-4-one (21). The title compound was obtained according to Method C using **21a** (96 mg, 0.26 mmol), trifluoroacetic acid (0.20 mL, 2.56 mmol), triethylsilane (0.13 mL, 0.78 mmol) and trifluoromethanesulfuric acid (2 µL, 0.03 mmol) in anhydrous CH_2Cl_2 (6 mL) as pale yellow solids (40 mg, 43%), mp 162–165 °C. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 2.66 (t, *J* = 7.7 Hz, 2H), 2.91 (t, *J* = 7.7 Hz, 2H), 3.14 (t, *J* = 8.4 Hz, 2H), 4.07 (t, *J* = 8.4 Hz, 2H), 5.42 (s, 1H), 6.71 (s, 1H), 6.78 (m, 2H), 6.88 (d, *J* = 7.7 Hz, 1H), 6.96 (td, *J* = 2.2, 8.3 Hz, 1H), 7.03 (d, *J* = 4.6 Hz, 2H), 7.29 (m, 1H), 8.53 (d, *J* = 4.6 Hz, 2H). MS (ESI) *m/z* = 359 $[M+H]^+$.

8-[(4-Fluorophenyl)(pyridin-4-yl)methyl]-1,2,5,6-tetrahydro-4*H*-pyrrolo[3,2,1-*ij*]quinolin-4-one (22). The title compound was obtained according to Method C using **22a** (65 mg, 0.17 mmol), trifluoroacetic acid (0.13 mL, 1.74 mmol), triethylsilane (80 µL, 0.52 mmol) and trifluoromethanesulfuric acid (2 µL, 0.02 mmol) in anhydrous CH_2Cl_2 (5 mL) as pale yellow solids (45 mg, 74%), mp 155–157 °C. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 2.66 (t, *J* = 7.7 Hz, 2H), 2.90 (t, *J* = 7.7 Hz, 2H), 3.13 (t, *J* = 8.5 Hz, 2H), 4.07 (t, *J* = 8.5 Hz, 2H), 5.41 (s, 1H), 6.70 (s, 1H), 6.77 (s, 1H), 6.99–7.06 (m, 6H), 8.53 (dd, *J* = 1.6， 4.5 Hz, 2H). MS (ESI) *m/z* = 359 $[M+H]^+$.

8-[(3-Chlorophenyl)(pyridin-4-yl)methyl]-1,2,5,6-tetrahydro-4*H*-pyrrolo[3,2,1-*ij*]quinolin-4-one (23). The title compound was obtained according to Method C using **23a** (85 mg, 0.22 mmol), trifluoroacetic acid (0.17 mL, 2.20 mmol), triethylsilane (0.11 mL, 0.65 mmol) and trifluoromethanesulfuric acid (2 µL, 0.02 mmol) in anhydrous CH_2Cl_2 (5 mL) as pale yellow solids (42 mg, 51%), mp 163–165 °C. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 2.66 (t, *J* = 7.7 Hz, 2H), 2.91 (t, *J* = 7.7 Hz, 2H), 3.14 (t, *J* = 8.4 Hz, 2H), 4.08 (t, *J* = 8.4 Hz, 2H), 5.40 (s, 1H), 6.70 (s, 1H), 6.77 (s, 1H), 6.98 (m, 1H), 7.02 (d, *J* = 4.6 Hz, 2H), 7.07 (s, 1H), 7.25 (m, 2H), 8.54 (d, *J* = 4.6 Hz, 2H). MS (ESI) *m/z* = 375 M^+.

8-[(4-Chlorophenyl)(pyridin-4-yl)methyl]-1,2,5,6-tetrahydro-4*H*-pyrrolo[3,2,1-*ij*]quinolin-4-one (24). The title compound was obtained according to Method C using **24a** (95 mg, 0.24 mmol), trifluoroacetic acid (0.19 mL, 2.43 mmol), triethylsilane (0.12 mL, 0.73 mmol) and trifluoromethanesulfuric acid (3 µL, 0.02 mmol) in anhydrous CH_2Cl_2 (5 mL) as pale yellow solids (56 mg, 62%), mp 136–138 °C. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 2.66 (t, *J* = 7.7 Hz, 2H), 2.90 (t, *J* = 7.7 Hz, 2H), 3.13 (t, *J* = 8.4 Hz, 2H), 4.07 (t, *J* = 8.4 Hz, 2H), 5.40 (s, 1H), 6.69 (s, 1H), 6.76 (s, 1H), 7.02 (m, 4H), 7.28 (d, *J* = 8.5 Hz, 2H), 8.53 (d, *J* = 4.6 Hz, 2H). MS (ESI) *m/z* = 375 M^+.

8-[(3-Methylphenyl)(pyridin-4-yl)methyl]-1,2,5,6-tetrahydro-4*H*-pyrrolo[3,2,1-*ij*]quinolin-4-one (25). The title compound was obtained according to Method C using **25a** (65 mg, 0.18 mmol), trifluoroacetic acid

(0.14 mL, 1.80 mmol), triethylsilane (0.85 µL, 0.53 mmol) and trifluoromethanesulfuric acid (2 µL, 0.02 mmol) in anhydrous CH_2Cl_2 (4 mL) as pale yellow solids (32 mg, 50%), mp 180–182 °C. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 2.31 (s, 3H), 2.66 (t, *J* = 7.7 Hz, 2H), 2.90 (t, *J* = 7.7 Hz, 2H), 3.13 (t, *J* = 8.4 Hz, 2H), 4.07 (t, *J* = 8.4 Hz, 2H), 5.39 (s, 1H), 6.72 (s, 1H), 6.79 (s, 1H), 6.87 (d, *J* = 7.7 Hz, 1H), 6.91 (s, 1H), 7.04 (d, *J* = 4.6 Hz, 2H), 7.07 (d, *J* = 7.5 Hz, 1H), 7.20 (t, *J* = 7.6 Hz, 1H), 8.51 (d, *J* = 4.6 Hz, 2H). MS (ESI) *m/z* = 355 $[M+H]^+$.

8-[((3,5-Bis(trifluoromethyl)phenyl)(pyridin-4-yl)methyl]-1,2,5,6-tetrahydro-4*H*-pyrrolo[3,2,1-*ij*]quinolin-4-one (26). The title compound was obtained according to Method C using **26a** (95 mg, 0.19 mmol), trifluoroacetic acid (0.15 mL, 1.93 mmol), triethylsilane (0.93 µL, 0.58 mmol) and trifluoromethanesulfuric acid (2 µL, 0.02 mmol) in anhydrous CH_2Cl_2 (6 mL) as pale yellow solids (38 mg, 42%), mp 182–184 °C. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 2.68 (t, *J* = 7.7 Hz, 2H), 2.92 (t, *J* = 7.7 Hz, 2H), 3.15 (t, *J* = 8.4 Hz, 2H), 4.09 (t, *J* = 8.4 Hz, 2H), 5.55 (s, 1H), 6.68 (s, 1H), 6.75 (s, 1H), 7.00 (d, *J* = 4.6 Hz, 2H), 7.54 (s, 2H), 7.80 (s, 1H), 8.58 (d, *J* = 4.6 Hz, 2H). MS (ESI) *m/z* = 477 $[M+H]^+$.

Method D: Wittig Reaction

To a suspension of methyltriphenylphosphonium bromide (2.2 equiv) in anhydrous THF (5 mL) was added n-BuLi (2.0 equiv) slowly under an atmosphere of nitrogene at –78 °C. The reaction mixture was stirred at room temperature for 1 h, and then cooled to –78 °C again followed by addition of a solution of a ketone (1.0 equiv) in THF (5 mL). The resulting mixture was stirred at ambient temperature for 5 h, and subsequently quenched with aqueous NH_4Cl, extracted with ethyl acetate (3 x 10 mL), dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by flash chromatograph on silica gel to yield the corresponding methylene compound.

8-(1-pyridin-3-ylvinyl)-1,2,5,6-tetrahydro-4*H*-pyrrolo[3,2,1-*ij*]quinolin-4-one (3). The title compound was obtained according to Method D using **5b** (200 mg, 0.72 mmol), methyltriphenylphosphonium bromide (564 mg, 1.58 mmol), and n-BuLi (2.5 M in hexanes, 0.58 mL, 1.44 mmol). The crude product was purified by flash chromatography column on silica gel (methanol/dichloromethane, 0 to 1:70) to give offwhite solids (117 mg, 59%), mp 97–99 °C. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 2.67 (t, *J* = 7.7 Hz, 2H), 2.95 (t, *J* = 7.7 Hz, 2H), 3.17 (t, *J* = 8.5 Hz, 2H), 4.10 (t, *J* = 8.5 Hz, 2H), 5.40 (s, 1H), 5.48 (s, 1H), 6.95 (s, 1H), 7.03 (s, 1H), 7.27 (dd, *J* = 4.9, 7.9 Hz, 1H), 7.61 (dt, *J* = 2.1, 7.9 Hz, 1H), 8.56 (dd, ^3J = 1.6, 4.8 Hz, 1H), 8.60 (d, *J* = 1.6 Hz, 1H). MS (ESI) *m/z* = 277 $[M+H]^+$.

8-(1-pyridin-4-ylvinyl)-1,2,5,6-tetrahydro-4*H*-pyrrolo[3,2,1-*ij*]quinolin-4-one (12). The title compound was obtained according to Method D using **13b** (200 mg, 0.72 mmol), methyltriphenylphosphonium bromide (564 mg, 1.58 mmol), and n-BuLi (2.5 M in hexanes, 0.58 mL, 1.44 mmol). The crude product was purified by flash chromatography column on silica gel (methanol/dichloromethane, 0 to 1:50) to give pale yellow semisolids (115 mg, 58%). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 2.69 (t, *J* = 7.7 Hz, 2H), 2.95 (t, *J* = 7.7 Hz, 2H), 3.18 (t, *J* = 8.5 Hz, 2H), 4.10 (t, *J* = 8.5 Hz, 2H), 5.51 (d, *J* = 8.1 Hz, 2H), 6.93 (s, 1H), 7.00 (s, 1H), 7.23 (dd, *J* = 1.6, 4.5 Hz, 2H), 8.58 (dd, *J* = 1.5, 4.6 Hz, 2H). MS (ESI) *m/z* = 277 $[M+H]^+$.

Method E: Catalytic hydrogenation

To a solution of a methylene compound (1 equiv) in methanol (8 mL) was added 5% Pd/C (15% weight). Subsequently, the reaction was stirred under a hydrogen atmosphere (maintained with balloons) for 10 h followed by filtration through a Celite cake and concentration in vacuo. The crude product was purified by flash chromatography on silica gel to yield the corresponding saturated product.

8-(1-Pyridin-3-ylethyl)-1,2,5,6-tetrahydro-4*H*-pyrrolo[3,2,1-*ij*]quinolin-4-one (2). The title compound was obtained according to Method E using **3** (59 mg, 0.21 mmol), and 5% Pd/C (9 mg). The crude product was purified by flash chromatography column on silica gel (methanol/dichloromethane, 0 to 1:50) to give white solids (36 mg, 62%), 100–102 °C. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 1.64 (d, J = 7.2 Hz, 3H), 2.65 (t, J = 7.8 Hz, 2H), 2.92 (t, J = 7.8 Hz, 2H), 3.14 (t, J = 8.4 Hz, 2H), 4.06 (t, J = 8.4 Hz, 2H), 4.11 (q, J = 7.2 Hz, 1H), 6.83 (s, 1H), 6.91 (s, 1H), 7.21 (dd, J = 4.7, 7.9 Hz, 1H), 7.49 (dt, J = 1.8, 7.9 Hz, 1H), 8.44 (dd, J = 1.5, 4.7 Hz, 1H), 8.51 (d, J = 2.2 Hz, 1H). MS (ESI) m/z = 279 $[M+H]^+$.

8-(1-Pyridin-4-ylethyl)-1,2,5,6-tetrahydro-4*H*-pyrrolo[3,2,1-*ij*]quinolin-4-one (11). The title compound was obtained according to Method E using **12** (115 mg, 0.38 mmol), and 5% Pd/C (16 mg). The crude product was purified by flash chromatography column on silica gel (methanol/dichloromethane, 0 to 1:50) to give offwhite solids (84 mg, 79%), 103–105 °C. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 1.61 (d, J = 7.2 Hz, 3H), 2.66 (t, J = 7.8 Hz, 2H), 2.92 (t, J = 7.8 Hz, 2H), 3.14 (t, J = 8.4 Hz, 2H), 4.06 (m, 3H), 6.81 (s, 1H), 6.90 (s, 1H), 7.13 (dd, J = 1.3, 4.8 Hz, 2H), 8.50 (dd, J = 1.3, 4.8 Hz, 2H). MS (ESI) m/z = 279 $[M+H]^+$.

Method F: Sodium Borohydride Reduction

To a solution of a ketone (1.0 equiv) in methanol (8 mL) was added sodium borohydride (2.0 equiv) at 0 °C. The mixture was stirred at the same temperature for 1 h before concentration in vacuo. The resulting solid was washed with water and diethylether in sequence to yield the corresponding alcohol.

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Supporting Information

1. The experimental details and characterization of compounds 4, 15 and 16 and intermediates 5a–9a, 13a, 14a and 18a–26a, as well as the ^{13}C-NMR of all final compounds.

Method A: Friedel-Crafts Acylation

1,2,5,6-Tetrahydro-4*H*-pyrrolo[3,2,1-*ij*]quinolin-4-one (1.0 equiv), nicotinoyl chloride hydrochloride or isonicotinoyl chloride hydrochloride (1.5 equiv) and $AlCl_3$ (5.0 equiv) were melted at 140 °C for 4 h followed by being cooled to 0 °C. A mixture of ice/water was added to decompose the excessive $AlCl_3$, and the resulting mixture was stirred at ambient temperature for 1 h. Extraction with $CHCl_3$ (3 x 20 mL) gave the organic layers, which were combined and dried over $MgSO_4$. After removal of solvent *in vacuo*, the residue was purified by flash chromatography on silica gel to yield the corresponding ketones.

Method B: Grignard Addition

To a solution of a ketone (1.0 equiv) in anhydrous THF was added dropwise a solution of an appropriate Grignard reagent (2.0–3.0 equiv) under an atmosphere of nitrogene at –78 °C. The reaction was stirred at the same temperature for another 1h, and subsequently warmed to room temperature. After being stirred for 15 h, the reaction was quenched with saturated aqueous $NHCl_4$ (5 mL), extracted with ethyl acetate (3 x 10 mL), dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash chromatograph on silica gel (methanol/dichloromethane, 0 to 1:30) to yield the corresponding alcohol.

8-[Hydroxy(phenyl)pyridin-4-ylmethyl]-1,2,5,6-tetrahydro-4*H*-pyrrolo[3,2,1-*ij*]quinolin-4-one (16). The title compound was obtained according to Method B using **13b** (200 mg, 0.72 mmol), phenylmagnesium bromide (1 M in THF, 2.16 mL, 2.16 mmol) in anhydrous THF (5 mL) as pale yellow solids (120 mg, 47%), mp 196–197 °C. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 2.63 (t, *J* = 7.7 Hz, 2H), 2.88 (t, *J* = 7.7 Hz, 2H), 3.11 (t, *J* = 8.3 Hz, 2H), 3.53 (s, br, 1H), 4.04 (t, *J* = 8.3 Hz, 2H), 6.88 (s, 1H), 6.93 (s, 1H), 7.25–7.33 (m, 7H), 8.49 (d, *J* = 3.2 Hz, 2H). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 24.4, 27.7, 31.5, 45.4, 81.2, 119.5, 122.6, 123.1, 125.3, 127.7, 127.8, 128.2, 128.7, 140.9, 141.4, 145.7, 149.4, 155.6, 167.6. MS (ESI) *m/z* = 357 $[M+H]^+$.

8-[Hydroxy(phenyl)pyridin-3-ylmethyl]-1,2,5,6-tetrahydro-4*H*-pyrrolo[3,2,1-*ij*]quinolin-4-one (5a). The title compound was obtained according to Method B using **5b** (200 mg, 0.72 mmol), phenylmagnesium bromide (1 M in THF, 2.16 mL, 2.16 mmol) in anhydrous THF (5 mL) as yellow solids (60 mg, 23%). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 2.66 (t, *J* = 7.7 Hz, 2H), 2.80 (s, 1H), 2.90 (t, *J* = 7.7 Hz, 2H), 3.13 (t, *J* = 8.5 Hz, 2H), 4.08 (t, *J* = 8.5 Hz, 2H), 6.90 (s, 1H), 6.95 (s, 1H), 7.27 (m, 3H), 7.32–7.37 (m, 3H), 7.67 (dt, *J* = 2.0, 8.0 Hz, 1H), 8.53 (dd, *J* = 1.6, 4.8 Hz, 1H), 8.55 (d, *J* = 2.3 Hz, 1H).

8-[Hydroxy(2-methoxyphenyl)pyridin-3-ylmethyl]-1,2,5,6-tetrahydro-4*H*-pyrrolo[3,2,1-*ij*]quinolin-4-one (6a). The title compound was obtained according to Method B using **6b** (200 mg, 0.72 mmol), 2-methoxyphenylmagnesium bromide (1 M in THF, 1.44 mL, 1.44 mmol) in anhydrous THF (10 mL) as yellow solids (58 mg, 21%). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 2.67 (t, *J* = 7.7 Hz, 2H), 2.90 (t, *J* = 7.7 Hz, 2H), 3.13 (t, *J* = 8.4 Hz, 2H), 3.72 (s, 3H), 4.08 (t, *J* = 8.5 Hz, 2H), 5.33 (s, 1H), 6.55 (dd, *J* = 1.6, 7.6 Hz, 1H), 6.88 (m, 2H), 6.90 (s, 1H), 6.98 (d, *J* = 8.0 Hz, 1H), 7.25 (m, 1H), 7.33 (m, 1H), 7.62 (dt, *J* = 1.9, 7.9 Hz, 1H), 8.40 (d, *J* = 1.9 Hz, 1H), 8.53 (dd, *J* = 1.6, 4.7 Hz, 1H).

8-[Hydroxy(3-methylphenyl)pyridin-3-ylmethyl]-1,2,5,6-tetrahydro-4*H*-pyrrolo[3,2,1-*ij*]quinolin-4-one (7a). The title compound was obtained according to Method B using **7b** (200 mg, 0.72 mmol), m-tolylmagnesium chloride (1 M in THF, 1.44 mL, 1.44 mmol) in anhydrous THF (10 mL) as yellow solids (62 mg, 23%). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 2.33 (s, 3H), 2.67 (t, *J* = 7.8 Hz, 2H), 2.77 (s, 1H), 2.91 (t, *J* = 7.8 Hz, 2H), 3.13 (t, *J* = 8.4 Hz, 2H), 4.08 (t, *J* = 8.5 Hz, 2H), 6.90 (s, 1H), 6.94 (s, 1H), 7.00 (d, *J* = 7.9 Hz, 1H), 7.10 (s, 1H), 7.13 (m, 1H), 7.22 (m , 1H), 7.27 (m, 1H), 7.67 (dt, *J* = 2.0, 8.0 Hz, 1H), 8.53 (dd, *J* = 1.5, 4.7 Hz, 1H), 8.54 (d, *J* = 1.8 Hz, 1H).

8-[(3-Chlorophenyl)(hydroxy)pyridin-3-ylmethyl]-1,2,5,6-tetrahydro-4*H*-pyrrolo[3,2,1-*ij*]quinolin-4-one (8a). The title compound was obtained according to Method B using **8b** (200 mg, 0.72 mmol), 3-chlorophenylmagnesium bromide (0.5 M in THF, 2.88 mL, 1.44 mmol) in anhydrous THF (10 mL) as yellow solids (72 mg, 26%). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 2.67 (t, *J* = 7.8 Hz, 2H), 2.82 (s, 1H), 2.91 (t, *J*

= 7.8 Hz, 2H), 3.14 (t, *J* = 8.5 Hz, 2H), 4.09 (t, *J* = 8.5 Hz, 2H), 6.86 (s, 1H), 6.92 (s, 1H), 7.15 (dt, *J* = 1.9, 7.2 Hz, 1H), 7.29 (m , 3H), 7.34 (m, 1H), 7.65 (dt, *J* = 2.0, 8.0 Hz, 1H), 8.55 (m, 2H).

8-[(4-Fluorophenyl)(hydroxy)pyridin-3-ylmethyl]-1,2,5,6-tetrahydro-4*H*-pyrrolo[3,2,1-*ij*]quinolin-4-one (9a). The title compound was obtained according to Method B using **9b** (300 mg, 1.08 mmol), 4-fluorophenylmagnesium bromide (0.8 M in THF, 2.69 mL, 2.16 mmol) in anhydrous THF (10 mL) as tan solids (128 mg, 34%). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 2.67 (t, *J* = 7.8 Hz, 2H), 2.78 (s, 1H), 2.91 (t, *J* = 7.7 Hz, 2H), 3.14 (t, *J* = 8.5 Hz, 2H), 4.08 (t, *J* = 8.5 Hz, 2H), 6.87 (s, 1H), 6.92 (s, 1H), 7.03 (m, 2H), 7.24–7.29 (m, 3H), 7.66 (dt, *J* = 2.0, 8.0 Hz, 1H), 8.54 (m, 2H).

8-(1-Hydroxy-2-methyl-1-pyridin-3-ylpropyl)-1,2,5,6-tetrahydro-4*H*-pyrrolo[3,2,1-*ij*]quinolin-4-one (13a). The title compound was obtained according to Method B using **13b** (150 mg, 0.54 mmol), isopropylmagnesium chloride (2.0 M in THF, 0.54 mL, 1.08 mmol) in anhydrous THF (10 mL) as yellow solids (124 mg, 36%). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 0.85 (d, *J* = 6.7 Hz, 3H), 0.94 (d, *J* = 6.7 Hz, 3H), 2.05 (s, 1H), 2.65 (t, *J* = 7.8 Hz, 2H), 2.83 (sept, 1H), 2.93 (t, *J* = 7.7 Hz, 2H), 3.15 (t, *J* = 8.4 Hz, 2H), 4.05 (t, *J* = 8.5 Hz, 2H), 7.13 (s, 1H), 7.21 (s, 1H), 7.38 (dd, *J* = 1.6, 4.7 Hz, 2H), 8.51 (dd, *J* = 1.5, 4.7 Hz, 2H).

8-[Cyclohexyl(hydroxy)pyridin-3-ylmethyl]-1,2,5,6-tetrahydro-4*H*-pyrrolo[3,2,1-*ij*]quinolin-4-one (14a). The title compound was obtained according to Method B using **13b** (200 mg, 0.72 mmol), cyclohexylmagnesium chloride (1.3 M in THF/Toluene, 1.11 mL, 1.44 mmol) in anhydrous THF (10 mL) as pale yellow solids (150 mg, 56%). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 1.06–1.16 (m, 3H), 1.25–1.36 (m, 2H), 1.42 (m, 1H), 1.64–1.81 (m, 4H), 2.07 (s, 1H), 2.37 (m, 1H), 2.65 (t, *J* = 7.7 Hz, 2H), 2.93 (t, *J* = 7.8 Hz, 2H), 3.16 (t, *J* = 8.4 Hz, 2H), 4.05 (t, *J* = 8.4 Hz, 2H), 7.11 (s, 1H), 7.19 (s, 1H), 7.37 (dd, *J* = 1.6, 4.6 Hz, 2H), 8.51 (dd, *J* = 1.5, 4.7 Hz, 2H).

8-[Hydroxy(2-methoxyphenyl)pyridin-3-ylmethyl]-1,2,5,6-tetrahydro-4*H*-pyrrolo[3,2,1-*ij*]quinolin-4-one (18a). The title compound was obtained according to Method B using **18b** (200 mg, 0.72 mmol), 2-methoxyphenylmagnesium bromide (1.0 M in THF, 1.44 mL, 1.44 mmol) in anhydrous THF (10 mL) as tan solids (77 mg, 28%). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 2.67 (t, *J* = 7.7 Hz, 2H), 2.90 (t, *J* = 7.8 Hz, 2H), 3.13 (t, *J* = 8.4 Hz, 2H), 3.69 (s, 3H), 4.08 (t, *J* = 8.5 Hz, 2H), 5.27 (s, 1H), 6.57 (dd, *J* = 1.6, 7.7 Hz, 1H), 6.88 (m, 3H), 6.98 (d, *J* = 8.2 Hz, 1H), 7.18 (dd, *J* = 1.6, 4.6 Hz, 2H), 7.34 (m, 1H), 8.55 (dd, *J* = 1.5, 4.6 Hz, 2H).

8-[Hydroxy(3-methoxyphenyl)pyridin-3-ylmethyl]-1,2,5,6-tetrahydro-4*H*-pyrrolo[3,2,1-*ij*]quinolin-4-one (19a). The title compound was obtained according to Method B using **19b** (200 mg, 0.72 mmol), 3-methoxyphenylmagnesium bromide (1.0 M in THF, 1.44 mL, 1.44 mmol) in anhydrous THF (10 mL) as yellow solids (145 mg, 52%). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 2.66 (t, *J* = 7.7 Hz, 2H), 2.88 (s, 1H), 2.90 (t, *J* = 7.8 Hz, 2H), 3.13 (t, *J* = 8.5 Hz, 2H), 3.77 (s, 3H), 4.08 (t, *J* = 8.5 Hz, 2H), 6.81 (m, 1H), 6.85 (m, 2H), 6.88 (s, 1H), 6.93 (s, 1H), 7.25 (m, 1H), 7.28 (dd, *J* = 1.6, 4.6 Hz, 2H), 8.56 (dd, *J* = 1.6, 4.6 Hz, 2H).

8-[Hydroxy(4-methoxyphenyl)pyridin-3-ylmethyl]-1,2,5,6-tetrahydro-4*H*-pyrrolo[3,2,1-*ij*]quinolin-4-one (20a). The title compound was obtained according to Method B using **20b** (200 mg, 0.72 mmol), 4-methoxyphenylmagnesium bromide (1.0 M in THF, 1.44 mL, 1.44 mmol) in anhydrous THF (10 mL) as tan solids (102 mg, 36%). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 2.67 (t, *J* = 7.7 Hz, 2H), 2.69 (s, 1H), 2.91 (t, *J* = 7.7

Hz, 2H), 3.13 (t, *J* = 8.5 Hz, 2H), 3.82 (s, 3H), 4.08 (t, *J* = 8.5 Hz, 2H), 6.86 (dd, *J* = 2.1, 6.8 Hz, 2H), 6.90 (s, 1H), 6.94 (s, 1H), 7.15 (dd, *J* = 2.1, 6.8 Hz, 2H), 7.27 (dd, *J* = 1.7, 4.6 Hz, 2H), 8.57 (dd, *J* = 1.6, 4.6 Hz, 2H).

8-[(3-Fluorophenyl)(hydroxy)pyridin-3-ylmethyl]-1,2,5,6-tetrahydro-4*H*-pyrrolo[3,2,1-*ij*]quinolin-4-one (21a). The title compound was obtained according to Method B using **21b** (200 mg, 0.72 mmol), 3-fluorophenylmagnesium bromide (1.0 M in THF, 1.44 mL, 1.44 mmol) in anhydrous THF (10 mL) as yellow solids (98 mg, 36%). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 2.67 (t, *J* = 7.7 Hz, 2H), 2.77 (s, 1H), 2.91 (t, *J* = 7.7 Hz, 2H), 3.14 (t, *J* = 8.4 Hz, 2H), 4.09 (t, *J* = 8.5 Hz, 2H), 6.86 (s, 1H), 6.91 (s, 1H), 7.02–7.06 (m, 3H), 7.27 (dd, *J* = 1.7, 4.6 Hz, 2H), 7.32 (m, 1H), 8.59 (d, *J* = 1.6, 4.6 Hz, 2H).

8-[(4-Fluorophenyl)(hydroxy)pyridin-3-ylmethyl]-1,2,5,6-tetrahydro-4*H*-pyrrolo[3,2,1-*ij*]quinolin-4-one (22a). The title compound was obtained according to Method B using **22b** (200 mg, 0.72 mmol), 4-fluorophenylmagnesium bromide (0.8 M in THF, 2.7 mL, 2.16 mmol) in anhydrous THF (5 mL) as orange solids (66 mg, 24%). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 2.67 (t, *J* = 7.8 Hz, 2H), 2.77 (s, 1H), 2.90 (t, *J* = 7.8 Hz, 2H), 3.14 (t, *J* = 8.5 Hz, 2H), 4.08 (t, *J* = 8.5 Hz, 2H), 6.86 (s, 1H), 6.91 (s, 1H), 7.03 (m, 2H), 7.24 (m, 4H), 8.57 (d, *J* = 1.6, 4.6 Hz, 2H).

8-[(3-Chlorophenyl)(hydroxy)pyridin-3-ylmethyl]-1,2,5,6-tetrahydro-4*H*-pyrrolo[3,2,1-*ij*]quinolin-4-one (23a). The title compound was obtained according to Method B using **23b** (200 mg, 0.72 mmol), 3-fluorophenylmagnesium bromide (0.5 M in THF, 2.88 mL, 1.44 mmol) in anhydrous THF (10 mL) as tan solids (85 mg, 30%). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 2.67 (t, *J* = 7.8 Hz, 2H), 2.80 (s, 1H), 2.91 (t, *J* = 7.8 Hz, 2H), 3.14 (t, *J* = 8.5 Hz, 2H), 4.09 (t, *J* = 8.5 Hz, 2H), 6.84 (s, 1H), 6.90 (s, 1H), 7.15 (dt, *J* = 1.6, 7.3 Hz, 1H), 7.29 (m, 5H), 8.59 (dd, *J* = 1.6, 4.7 Hz, 2H).

8-[(4-Chlorophenyl)(hydroxy)pyridin-3-ylmethyl]-1,2,5,6-tetrahydro-4*H*-pyrrolo[3,2,1-*ij*]quinolin-4-one (24a). The title compound was obtained according to Method B using **24b** (200 mg, 0.72 mmol), 3-fluorophenylmagnesium bromide (0.5 M in THF, 2.88 mL, 1.44 mmol) in anhydrous THF (10 mL) as tan solids (85 mg, 30%). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 2.67 (t, *J* = 7.8 Hz, 2H), 2.74 (s, 1H), 2.91 (t, *J* = 7.8 Hz, 2H), 3.14 (t, *J* = 8.5 Hz, 2H), 4.08 (t, *J* = 8.5 Hz, 2H), 6.85 (s, 1H), 6.90 (s, 1H), 7.22 (m, 2H), 7.25 (dd, *J* = 1.6, 4.6 Hz, 2H), 7.32 (m, 2H), 8.58 (dd, *J* = 1.5, 4.6 Hz, 2H).

8-[Hydroxy(3-methylphenyl)pyridin-3-ylmethyl]-1,2,5,6-tetrahydro-4*H*-pyrrolo[3,2,1-*ij*]quinolin-4-one (25a). The title compound was obtained according to Method B using **25b** (200 mg, 0.72 mmol), m-tolylmagnesium chloride (1.0 M in THF, 1.44 mL, 1.44 mmol) in anhydrous THF (10 mL) as tan solids (65 mg, 24%). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 2.33 (s, 3H), 2.67 (t, *J* = 7.8 Hz, 2H), 2.74 (s, 1H), 2.91 (t, *J* = 7.7 Hz, 2H), 3.14 (t, *J* = 8.4 Hz, 2H), 4.08 (t, *J* = 8.4 Hz, 2H), 6.89 (s, 1H), 6.94 (s, 1H), 7.00 (d, *J* = 7.6 Hz, 1H), 7.09 (s, 1H), 7.14 (d, *J* = 7.3 Hz, 1H), 7.23 (t, *J* = 7.7 Hz, 1H), 7.27 (dd, *J* = 1.6, 4.6 Hz, 2H), 8.57 (dd, *J* = 1.6, 4.6 Hz, 2H).

8-{Hydroxy(pyridin-3-yl)[3,5-bis(trifluoromethyl)phenyl]methyl}-1,2,5,6-tetrahydro-4*H*-pyrrolo[3,2,1-*ij*]quinolin-4-one (26a). The title compound was obtained according to Method B using **26b** (200 mg, 0.72 mmol), 3,5-bis(trifluoromethyl)phenylmagnesium bromide (0.5 M in THF, 2.88 mL, 1.44

mmol) in anhydrous THF (12 mL) as yellow solids (96 mg, 27%). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 2.68 (t, *J* = 7.8 Hz, 2H), 2.91 (t, *J* = 7.8 Hz, 2H), 2.99 (s, 1H),3.15 (t, *J* = 8.5 Hz, 2H), 4.10 (t, *J* = 8.5 Hz, 2H), 6.79 (s, 1H), 6.84 (s, 1H), 7.25 (dd, *J* = 1.6, 4.6 Hz, 2H), 7.84 (s, 3H), 8.62 (dd, *J* = 1.6, 4.6 Hz, 2H).

Method C: Triethylsilane Reduction

To an alcohol (1.0 equiv) in anhydrous dichloromethane was added in sequence by syringe trifluoroacetic acid (10 equiv), triethylsilane (3.0 equiv) and trifluoromethanesulfuric acid (0.1 equivalent) under an atmosphere of nitrogen at 0 °C. The resulting solution was stirred at room temperature for 18–48 h. Afterwards, the reaction mixture was separated, and the queous layer was extracted with dichloromethane (2 x 10 mL). The combined organic layers were washed with aqueous $NaHCO_3$ and brine, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash chromatograph on silica gel (methanol/dichloromethane, 0 to 1:40) to yield the corresponding dehydoxyl product.

8-(Pyridin-3-ylmethyl)-1,2,5,6-tetrahydro-4*H*-pyrrolo[3,2,1-*ij*]quinolin-4-one (1). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 24.4, 27.7, 31.6, 38.8, 45.3, 120.2, 123.4, 123.7, 125.9, 129.3, 135.2, 136.1, 136.7, 140.0, 147.7, 150.1, 167.4.

8-[Phenyl(pyridin-3-yl)methyl]-1,2,5,6-tetrahydro-4*H*-pyrrolo[3,2,1-*ij*]quinolin-4-one (5). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 24.4, 27.7, 31.6, 45.3, 54.2, 120.1, 123.2, 124.2, 126.5, 126.7, 128.6, 129.2, 129.2, 136.6, 138.2, 139.5, 140.2, 142.8, 147.8, 150.8, 167.5.

8-[(2-Methoxyphenyl)(pyridin-3-yl)methyl]-1,2,5,6-tetrahydro-4*H*-pyrrolo[3,2,1-*ij*]quinolin-4-one (6). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 24.4, 27.8, 31.6, 45.3, 47.1, 55.5, 110.7, 119.9, 120.4, 123.1, 124.3, 126.5, 128.0, 129.0, 130.0, 131.4, 136.5, 138.1, 139.6, 140.0, 147.4, 150.8, 156.8, 167.5.

8-[(3-Methylphenyl)(pyridin-3-yl)methyl]-1,2,5,6-tetrahydro-4*H*-pyrrolo[3,2,1-*ij*]quinolin-4-one (7). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 21.5, 24.4, 27.7, 31.6, 45.3, 54.2, 120.0, 123.2, 124.2, 126.2, 126.5, 127.5, 128.5, 129.2, 129.9, 136.6, 138.3, 138.4, 139.5, 140.1, 142.7, 147.7, 150.8, 167.5.

8-[(3-Chlorophenyl)(pyridin-4-yl)methyl]-1,2,5,6-tetrahydro-4*H*-pyrrolo[3,2,1-*ij*]quinolin-4-one (8). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 24.4, 27.7, 31.5, 45.3, 53.9, 120.2, 123.4, 124.2, 126.4, 127.0, 127.4, 129.3, 129.4, 129.8, 134.6, 136.5, 137.4, 138.7, 140.4, 144.9, 148.0, 150.7, 167.5.

8-[(4-Fluorophenyl)(pyridin-3-yl)methyl]-1,2,5,6-tetrahydro-4*H*-pyrrolo[3,2,1-*ij*]quinolin-4-one (9). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 24.4, 27.7, 31.5, 45.3, 53.4, 115.4 (d, $^2J_{C,F}$ = 21.1 Hz), 120.2, 123.3, 124.1, 126.4, 129.3, 130.6 (d, $^3J_{C,F}$ = 7.7 Hz), 136.5, 138.0, 138.5 (d, $^4J_{C,F}$ = 3.2 Hz), 139.3, 140.3, 147.9, 150.7, 161.6 (d, $^1J_{C,F}$ = 246 Hz), 167.5.

8-(Pyridin-4-ylmethyl)-1,2,5,6-tetrahydro-4*H*-pyrrolo[3,2,1-*ij*]quinolin-4-one (10). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 24.4, 27.7, 31.5, 41.0, 45.3, 120.3, 123.9, 124.0, 126.1, 129.4, 134.2, 140.1, 149.9, 150.2, 167.4.

8-(2-Methyl-1-pyridin-4-ylpropyl)-1,2,5,6-tetrahydro-4*H*-pyrrolo[3,2,1-*ij*]quinolin-4-one (13). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 21.6, 21.7, 24.5, 27.8, 31.5, 31.6, 45.3, 60.1, 120.1, 122.7, 123.2, 125.1, 129.3,

138.6, 140.1, 149.9, 153.8, 167.4.

8-[Cyclohexyl(pyridin-3-yl)methyl]-1,2,5,6-tetrahydro-4*H*-pyrrolo[3,2,1-*ij*]quinolin-4-one (14). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 24.4, 26.1, 26.2, 26.3, 27.7, 31.5, 31.7, 32.0, 40.8, 45.2, 58.7, 120.1, 122.8, 123.3, 125.2, 129.2, 138.1, 140.0, 149.9, 153.4, 167.4.

8-[Phenyl(pyridin-4-yl)methyl]-1,2,5,6-tetrahydro-4*H*-pyrrolo[3,2,1-*ij*]quinolin-4-one (17). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 24.4, 27.7, 31.5, 45.3, 56.1, 120.1, 124.3, 124.5, 126.5, 126.9, 128.6, 129.2, 129.2, 137.6, 140.3, 142.2, 149.9, 152.9, 167.5.

8-[(2-Methoxyphenyl)(pyridin-4-yl)methyl]-1,2,5,6-tetrahydro-4*H*-pyrrolo[3,2,1-*ij*]quinolin-4-one (18). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 24.4, 27.7, 31.6, 45.3, 48.9, 55.5, 110.7, 119.9, 120.4, 124.4, 124.5, 126.6, 128.2, 129.0, 130.2, 130.8, 137.4, 140.1, 149.6, 153.3, 156.9, 167.5.

8-[(3-Methoxyphenyl)(pyridin-4-yl)methyl]-1,2,5,6-tetrahydro-4*H*-pyrrolo[3,2,1-*ij*]quinolin-4-one (19). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 24.4, 27.7, 31.5, 45.3, 55.2, 56.0, 111.6, 115.7, 120.1, 121.7, 124.2, 124.5, 126.5, 129.2, 129.6, 137.4, 140.3, 143.8, 149.9, 152.7, 159.8, 167.5.

8-[(4-Methoxyphenyl)(pyridin-4-yl)methyl]-1,2,5,6-tetrahydro-4*H*-pyrrolo[3,2,1-*ij*]quinolin-4-one (20). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 24.4, 27.7, 31.5, 45.3, 55.2, 55.2, 114.0, 120.1, 124.2, 124.4, 126.4, 129.2, 130.2, 134.3, 138.0, 140.2, 149.8, 153.2, 158.4, 167.5. MS (ESI) *m/z* = 371 $[M+H]^+$.

8-[(3-Fluorophenyl)(pyridin-4-yl)methyl]-1,2,5,6-tetrahydro-4*H*-pyrrolo[3,2,1-*ij*]quinolin-4-one (21). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 24.4, 27.7, 31.5, 45.3, 55.7, 113.9 (d, $^2J_{C,F}$ = 21.1 Hz), 113.2 (d, $^2J_{C,F}$ = 21.9 Hz), 120.2, 124.2, 124.4, 124.9 (d, $^4J_{C,F}$ = 2.6 Hz), 126.5, 129.4, 130.1 (d, $^3J_{C,F}$ = 8.2 Hz), 136.9, 140.5, 144.8 (d, $^3J_{C,F}$ = 6.6 Hz), 150.0, 152.2, 163.0 (d, $^1J_{C,F}$ = 247 Hz), 167.5. MS (ESI) *m/z* = 359 $[M+H]^+$.

8-[(4-Fluorophenyl)(pyridin-4-yl)methyl]-1,2,5,6-tetrahydro-4*H*-pyrrolo[3,2,1-*ij*]quinolin-4-one (22). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 24.4, 27.7, 31.5, 45.3, 55.3, 115.5 (d, $^2J_{C,F}$ = 21.6 Hz), 120.2, 124.2, 124.4, 126.4, 129.3, 130.7 (d, $^3J_{C,F}$ = 8.1 Hz), 137.4, 138.0 (d, $^4J_{C,F}$ = 3.0 Hz), 140.4, 150.0, 152.7, 161.7 (d, $^1J_{C,F}$ = 246 Hz), 167.5.

8-[(3-Chlorophenyl)(pyridin-4-yl)methyl]-1,2,5,6-tetrahydro-4*H*-pyrrolo[3,2,1-*ij*]quinolin-4-one (23). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 24.4, 27.7, 31.5, 45.4, 55.7, 120.3, 124.2, 124.4, 126.5, 127.2, 127.4, 129.3, 129.4, 129.9, 134.6, 136.8, 140.6, 144.3, 150.0, 152.0, 167.5.

8-[(4-Chlorophenyl)(pyridin-4-yl)methyl]-1,2,5,6-tetrahydro-4*H*-pyrrolo[3,2,1-*ij*]quinolin-4-one (24). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 24.4, 27.7, 31.5, 45.3, 55.4, 120.2, 124.2, 124.4, 126.4, 128.8, 129.4, 130.5, 132.8, 137.1, 140.5, 140.8, 150.0, 152.3, 167.5.

8-[(3-Methylphenyl)(pyridin-4-yl)methyl]-1,2,5,6-tetrahydro-4*H*-pyrrolo[3,2,1-*ij*]quinolin-4-one (25). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 21.5, 24.4, 27.7, 31.5, 45.3, 56.0, 120.1, 124.2, 124.5, 126.3, 126.5, 127.7, 128.5, 129.2, 129.9, 137.7, 138.3, 140.3, 142.1, 149.8, 153.0, 167.5.

8-[((3,5-Bis(trifluoromethyl)phenyl)(pyridin-4-yl)methyl]-1,2,5,6-tetrahydro-4*H*-pyrrolo[3,2,1-*ij*]quinolin-4-one (26). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 24.4, 27.7, 31.4, 45.4, 55.6, 56.0, 120.6, 121.2 (t,

$^{3}J_{C,F}$ = 3.64 Hz), 122.0, 124.2, 124.2, 125.3 (d, $^{1}J_{C,F}$ = 294 Hz), 129.2 (d, $^{4}J_{C,F}$ = 2.4 Hz), 129.9, 131.9 (q, $^{2}J_{C,F}$ = 33.2 Hz), 135.5, 141.1, 145.0, 150.4, 150.8, 167.5.

Method D: Wittig Reaction

To a suspension of methyltriphenylphosphonium bromide (2.2 equiv) in anhydrous THF (5 mL) was added n-BuLi (2.0 equiv) slowly under an atmosphere of nitrogene at –78 °C. The reaction mixture was stirred at room temperature for 1 h, and then cooled to –78 °C again followed by addition of a solution of a ketone (1.0 equiv) in THF (5 mL). The resulting mixture was stirred at ambient temperature for 5 h, and subsequently quenched with aqueous NH_4Cl, extracted with ethyl acetate (3 x 10 mL), dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by flash chromatograph on silica gel to yield the corresponding methylene compound.

8-(1-pyridin-3-ylvinyl)-1,2,5,6-tetrahydro-4*H*-pyrrolo[3,2,1-*ij*]quinolin-4-one (3). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 24.4, 27.6, 31.6, 45.4, 114.8, 119.9, 123.0, 123.2, 125.5, 129.1, 135.5, 136.1, 137.4, 141.5, 146.9, 149.0, 149.3, 167.5.

8-(1-pyridin-4-ylvinyl)-1,2,5,6-tetrahydro-4*H*-pyrrolo[3,2,1-*ij*]quinolin-4-one (12). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 24.3, 27.6, 31.6, 45.4, 115.9, 119.9, 122.8, 123.3, 125.6, 129.1, 135.5, 141.5, 147.9, 149.2, 149.9, 167.5.

Method E: Catalytic hydrogenation

To a solution of a methylene compound (1 equiv) in methanol (8 mL) was added 5% Pd/C (15% weight). Subsequently, the reaction was stirred under a hydrogen atmosphere (maintained with balloons) for 10 h followed by filtration through a Celite cake and concentration in vacuo. The crude product was purified by flash chromatography on silica gel to yield the corresponding saturated product.

8-(1-Pyridin-3-ylethyl)-1,2,5,6-tetrahydro-4*H*-pyrrolo[3,2,1-*ij*]quinolin-4-one (2). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 21.8, 24.5, 27.8, 31.6, 42.3, 45.3, 120.1, 122.4, 123.3, 124.7, 129.2, 134.7, 140.0, 140.7, 141.6, 147.6, 149.3, 167.4.

8-(1-Pyridin-4-ylethyl)-1,2,5,6-tetrahydro-4*H*-pyrrolo[3,2,1-*ij*]quinolin-4-one (11). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 21.3, 24.4, 27.7, 31.6, 44.1, 45.3, 120.1, 122.5, 122.8, 124.8, 129.2, 140.1, 149.9, 155.1, 167.5.

Method F: Sodium Borohydride Reduction

To a solution of a ketone (1.0 equiv) in methanol (8 mL) was added sodium borohydride (2.0 equiv) at 0 °C. The mixture was stirred at the same temperature for 1 h before concentration *in vacuo*. The resulting solid was washed with water and diethylether in sequence to yield the corresponding alcohol.

8-[Hydroxy(pyridin-3-yl)methyl]-1,2,5,6-tetrahydro-4*H*-pyrrolo[3,2,1-*ij*]quinolin-4-one (4). The title compound was obtained according to Method E using **5b** (200 mg, 0.72 mmol) and sodium borohydride (54 mg, 1.44 mmol) as offwhite solids (163 mg, 81%), 162–164 °C. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 2.63 (t, J = 7.8 Hz, 2H), 2.92 (t, J = 7.8 Hz, 2H), 3.13 (t, J = 8.5 Hz, 2H), 3.39 (s, br, 1H), 4.04 (t, J = 8.5 Hz, 2H), 5.80

(s, 1H), 6.99 (s, 1H), 7.06 (s, 1H), 7.25 (dd, J = 4.8, 7.8 Hz, 1H), 7.71 (dt, J = 1.5, 7.9 Hz, 1H), 8.44 (dd, J = 1.3, 4.7 Hz, 1H), 8.57 (d, J = 1.6 Hz, 1H). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 24.4, 27.7, 31.5, 45.4, 74.0, 120.2, 121.8, 123.4, 124.1, 129.4, 134.0, 138.8, 139.6, 141.1, 148.0, 148.6, 167.6. MS (ESI) *m/z* = 281 $[M+H]^+$.

8-[Hydroxy(pyridin-4-yl)methyl]-1,2,5,6-tetrahydro-4*H*-pyrrolo[3,2,1-*ij*]quinolin-4-one (15). The title compound was obtained according to Method E using **13b** (200 mg, 0.72 mmol) and sodium borohydride (55 mg, 1.44 mmol) as white solids (190 mg, 94%), 252–253 °C. ^{1}H-NMR (500 MHz, DMSO-d_6): δ 2.52 (t, J = 7.7 Hz, 2H), 2.87 (t, J = 7.7 Hz, 2H), 3.08 (t, J = 8.5 Hz, 2H), 3.90 (t, J = 8.5 Hz, 2H), 5.63 (d, J = 4.0 Hz, 1H), 6.00 (d, J = 4.0 Hz, 1H), 7.02 (s, 1H), 7.08 (s, 1H), 7.36 (dd, J = 1.6, 4.5 Hz, 2H), 8.46 (dd, J = 1.6, 4.5 Hz, 2H). ^{13}C-NMR (125 MHz, DMSO-d_6): δ 23.7, 27.0, 31.0, 44.9, 73.0, 119.6, 121.0, 121.2, 123.4, 128.7, 139.3, 140.3, 149.3, 154.3, 166.5. MS (ESI) *m/z* = 281 $[M+H]^+$.

2. HPLC purity control of all final compounds.

The Surveyor®-LC-system consisted of a pump, an autosampler, and a PDA detector. Mass spectrometry was performed on a TSQ® Quantum (Thermo Electron Corporation, Dreieich, Germany). The triple quadrupole mass spectrometer was equipped with an electrospray interface (ESI). The system was operated by the standard software Xcalibur®.

A RP C18 NUCLEODUR® 100-5 (125 × 3 mm) column (Macherey-Nagel GmbH, Duehren, Germany) was used as stationary phase. All solvents were HPLC grade.

In a gradient run the percentage of acetonitrile (containing 0.1% triflouro-acetic acid) in water was increased from an initial concentration of 3% at 0 min to 100% at 15 min and kept at 100% for 3 min.

The injection volume was 10 µl and flow rate was set to 350 µl/min. MS analysis was carried out at a spray voltage of 3800 V, a capillary temperature of 350 °C and a source CID of 10 V. Spectra were acquired in positive mode from 100 to 1000 m/z and full scan UV-mode. In some cases APC ionization had to be applied.

Compd.	RT (min)	Purity [%]
1	11.71	99.4%
2	12.29	99.7%
3	12.81	98.0%
4	9.11	99.6%
5	13.73	99.7%
6	12.56	98.0%
7	13.25	97.3%
8	13.48	96.2%
9	13.54	96.3%
10	11.70	98.6%
11	12.31	99.7%

Compd.	RT (min)	Purity [%]
12	10.81	98.9%
13	9.71	99.9%
14	11.51	99.9%
15	8.90	99.6%
16	13.82	99.6%
17	7.78	98.1%
18	12.59	98.3%
19	13.45	99.8%
20	12.08	99.7%
21	13.60	99.1%
22	13.81	97.5%
23	9.55	99.3%
24	12.54	99.7%
25	9.27	99.9%
26	14.97	99.6%

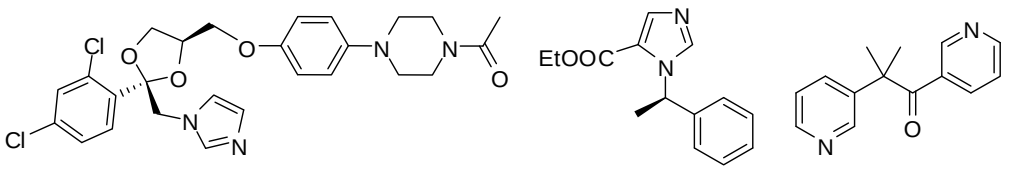
3.IV. Novel Imidazol-1-ylmethyl Substituted 1,2,5,6-Tetrahydro-pyrrolo[3,2,1-*ij*]quinolin-4-ones as Potent CYP11B1 Inhibitors for the Treatment of Cushing's Syndrome

Introduction

Cortisol is the principal human glucocorticoid exhibiting many important physiological functions, such as regulation of protein, carbohydrate and fat metabolism, it is counteracting insulin, maintaining blood pressure and cardiovascular function, and suppressing the immune system's inflammatory response. Cortisol is biosynthesized in the adrenal cortex with the final conversion of 11-deoxycortisol catalyzed by 11β-hydroxylase (CYP11B1). Normally, the production and secretion of cortisol are precisely controlled by adrenocorticotropic hormone (ACTH) within the negative feedback cycle of the hypothalamic-pituitary-adrenal axis. However, pathological changes in adrenal and upstream regulation can cause overproduction of cortisol. This disease state is termed hypercortisolism or Cushing's syndrome. In addition to symptoms like central obesity and physical or psychological discomfort such as fatigue, backache, headache, depression and anxiety in patients with hypercortisolism, overproduction of cortisol is also associated with a higher risk of hypertension and diabetes mellitus type II,[1] which in the long run leads to increased mortality.

Apart from surgical removal of some adrenal or pituitary tumors, CYP11B1 inhibition as a pharmacotherapeutic approach to block cortisol biosynthesis is an important alternative in the treatment of hypercortisolism. Currently, inhibitors of adrenal steroidogenesis employed clinically include ketoconazole (KET), etomidate (ETO) and metyrapone (MET) (Chart 1). Among them, the antifungal agent KET (inhibiting CYP11B1, CYP11B2, CYP17, and CYP11A1.) and the anesthetic ETO (inhibiting CYP11B1, CYP11B2, CYP11A1) are used as non-selective enzyme inhibitors. They are accompanied by severe side effects,[2] whereas MET is the unique one being mentioned as a relatively selective CYP11B1 inhibitor.[2]

Chart 1. Structures of drugs in clinic for the treatment of hypercortisolism.



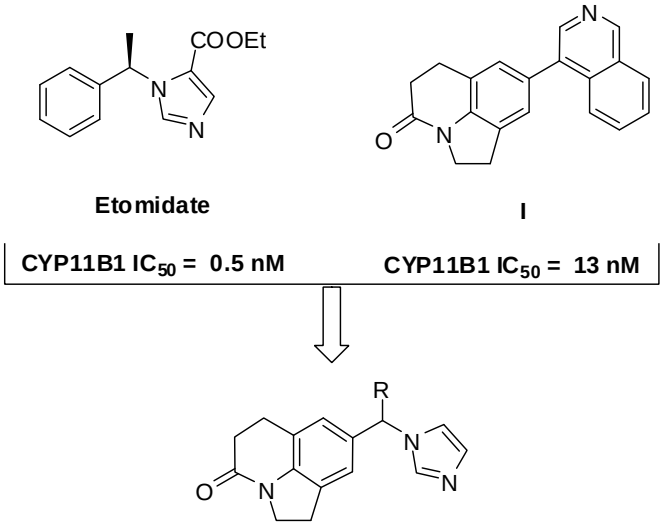
Ketoconazole Etomidate Metyrapone

Studies have demonstrated that MET can effectively reduce cortisol levels in patients with all types of Cushing's syndrome.[3] However, long-term administration of MET in high doses results in nausea, dizziness, skin rash, edema, acne, and hirsutism.[4] This absence of an effective and safe therapy for high cortisol levels encouraged us and others to design CYP11B1 inhibitors more potent and more selective CYP11B1 inhibitors.[5]

Many CYP inhibitors have been successfully designed based on a sp^2 hybrid nitrogen coordinating to the heme iron located as the center ion of the porphyrin moiety of CYP enzymes, e.g. inhibitors of CYP11B2,[6] CYP17[7] and CYP19[8]. This reversible inhibition manner is employed to design new CYP11B1 inhibitors with high potency. However, the selectivity is a challenging goal to achieve, especially over CYP11B2 due to high sequence homology of more than 93% shared between CYP11B1 and CYP11B2. Inhibition of other important steroidogenic enzymes, such as CYP17 and CYP19, should also be avoided because this would result in severe side effects. Recently, compounds originating from ETO have been reported to exhibit strong CYP11B1 inhibition.[5,9] Some of them also showed improved selectivity.[9]

In the present study, ETO (IC_{50} = 0.5 nM) was also used as a starting point as well as reference compound **I**[6e] (IC_{50} = 13 nM) to design novel inhibitors (Chart 2). The replacement of phenyl moiety of the ETO scaffold by the tetrahydropyrroloquinolinone group from reference compound **I** led to a series of imidazol-1-ylmethyl substituted 1,2,5,6-tetrahydro-pyrrolo[3,2,1-*ij*]quinolin-4-ones. It seemed reasonable that this hybrid would inherit the potent inhibition of CYP11B1. The introduction of the CH bridge between the tetrahydropyrroloquinolinone core and the N-containing heterocycle were deemed to be advantageous for the selectivity over CYP11B2. After optimization of the substituents on the CH bridge with various functional groups differing in electrostatic potential and bulkiness, such as aliphatic groups, substituted phenyl, and heteroaryls, the selectivity could be further improved. Besides evaluation of CYP11B1 inhibition and selectivity toward CYP11B2, the potent and selective compounds were further tested for selectivity against CYP17 and CYP19. Furthermore, after chiral separation the biological activities of *R*- and *S*-enantiomers

Chart 2. Drug design concept.

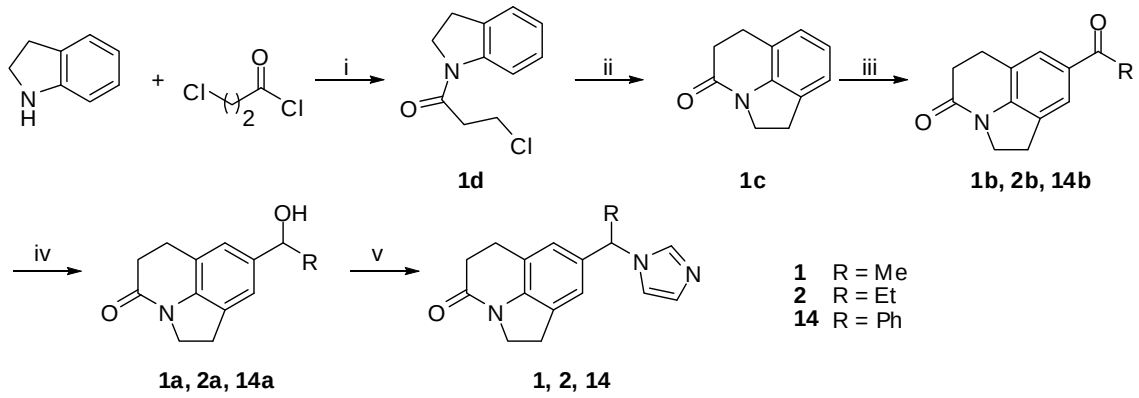


were investigated with compounds **4** and **7**, which to the most extent inclined to inhibit CYP11B1 or CYP11B2, respectively.

Results and Discussions

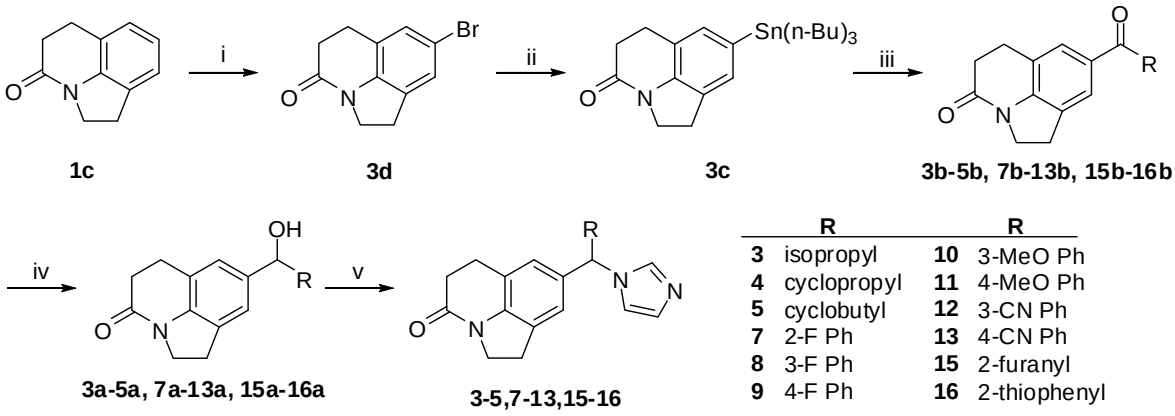
Chemistry. The syntheses of compounds **1**–**17** are shown in Schemes 1–3. A common synthetic route was exploited for the final compounds **1**–**5** and **7**–**16** (Schemes 1–2). The tetrahydropyrroloquinolinone core **1c**, as the common intermediate, was synthesized from commercially available 2,3-dihydro-1*H*-indole via N-acylation with 3-chloro-propionyl chloride followed by cyclization with $AlCl_3$ under melting conditions. **1c** was subsequently converted into substituted ketone intermediates, from which the desired final compounds were obtained after further reduction with sodium borohydride, and thereafter imidazolation with 1,1'-sulfonyldiimidazole in THF.[10] The ketone intermediates were prepared via two different approaches. Only for the introduction of acetyl (**1b)**, propanoyl (**2b)** and benzoyl (**14b)**, Friedel-Crafts acylation with the corresponding acyl chlorides gave the desired products (Scheme 1).[11] For the synthesis of the other

Scheme 1[a]



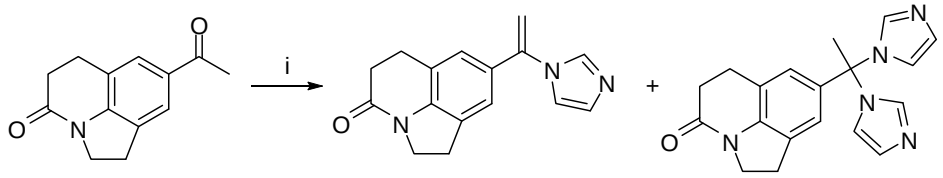
[a] Reagents and conditions: (i) pyridine, THF, 0 °C; (ii) $AlCl_3$, 140 °C; (iii) Method A: RCOCl, $AlCl_3$, CH_2Cl_2, reflux, then 6 N aq. HCl; (iv) Method B: $NaBH_4$, MeOH, 0 °C; (v) Method C: imidazole, $SOCl_2$, THF, room temp.

Scheme 2[a]



[a] Reagents and conditions: (i) NBS, DMF, 0 °C; (ii) Sn_2Bu_6, $Pd(PPh_3)_4$, toluene; (iii) Method D: RCOCl, $PdCl_2(PPh_3)_2$, toluene; (iv) Method B: $NaBH_4$, MeOH, 0 °C; (v) Method C: imidazole, $SOCl_2$, THF, room temp.

Scheme 3[a]



1b **6** **17**

[a] Reagents and conditions: (i) imidazole, $SOCl_2$, CH_2Cl_2, room temp.

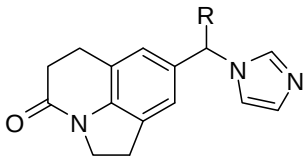
derivatives, the core was first brominated with NBS[12] and subsequently stannated with bis(tributyltin) to afford the key intermediate **3c**, which subsequently coupled with various acyl chlorides to give the corresponding ketones **3b**–**5b, 7b**–**13b** and **15b**–**16b** in 25–75% yields[13] (Scheme 2). In order to abrogate the chiral center, **1b** was reacted with an excess of 1,1'-sulfonyldiimidazole and imidazole in CH_2Cl_2 to give the vinyl imidazole analogue **6** as the minor product, and the diimidazolyl compound **17** as the major one. [14]

Inhibition of Human CYP11B1 and CYP11B2. The synthesized compounds were evaluated for their inhibitory activities in V79 MZh cells expressing either human CYP11B1 or CYP11B2.[15] IC_{50} values are presented in comparison to the reference compounds **I**, and etomidate and the CYP11B1 inhibitor metyrapone (Table 1).

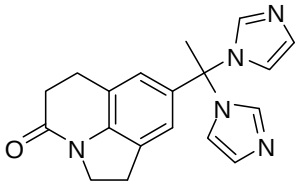
Most the compounds showed strong inhibition of CYP11B1, with IC_{50} values ranging from 1.4 to 110 nM. Analogues with aliphatic chains at the CH bridge (**1**–**6**, IC_{50} < 20 nM) exhibited a stronger inhibition than compounds substituted with aromatic rings (**7**–**17**, IC_{50} values ranging from 13 to 144 nM). For the aliphatic analogues **1**–**5** the size of the substituents exhibited a significant influence on the inhibitory potency. With increasing bulkiness of the aliphatic substituents were increasing (Me < Et < cyclopropyl < cyclobutyl < isopropyl),[16] the potency of the corresponding compounds increased from 17 nM (**1**, Me) to 1.4 nM (**3**, isopropyl). Thus, compound **3** turned out to be the most potent CYP11B1 inhibitor in this study, being ten-fold more potent than metyrapone (IC_{50} = 15 nM), the drug used in clinic. However, compound **3** also exhibited strong inhibition of CYP11B2 (IC_{50} = 3.8 nM), resulting in poor selectivity (SF = 2.7). On the contrary, compound **4** exceeded metyrapone both in terms of inhibitory potency (IC_{50} = 2.2 nM) and selectivity (SFs of 11 and 4.8 for **4** and metyrapone, respectively). Dehydrogenation of the methyl group to methylene (**6**) resulted in a similarly potent compound with improved selectivity (SF = 5.3) compared to **1**.

However, further modification by introducing the more bulky phenyl group onto the CH bridge (**14**) led to a slight decrease in inhibition (IC_{50} = 50 nM), which might be due to some steric hindrance. This hypothesis is substantiated by the fact that only F substitution (compounds **7**–**9**) led to similarly active compounds (IC_{50} ranging 27–40 nM), whereas analogues substituted with the more bulky MeO or CN (compounds **10**–**13**) mostly exhibited reduced inhibitory activity (IC_{50} ranging 40–144 nM). It certainly has also to be taken into account that MeO donates electrons, while CN is a strong electron withdrawing group. Moreover, the position of the substituent at the phenyl group is also important for inhibitory potency. It is obvious that analogues with *para*- substitution are more potent than the ones with *meta*- substitution, especially for MeO and CN, where the *para*- substituted analogues **11** and **13** (IC_{50} = 40 and 70 nM) are more than the

Table 1. Inhibition of CYP11B1 and CYP11B2 by compounds **1**–**17**.



1-16



17

Compd	R	CYP11B1[a] IC_{50} [b] (nM)	CYP11B2[c] IC_{50} [b] (nM)	S. F.[d]
1	Me	17	15	0.9
2	Et	6.5	5.8	0.9
3	*i*-propyl	1.4	3.8	2.7
4	*c*-propyl	2.2	24	11
5	*c*-butyl	3.4	4.2	1.2
6	$=CH_2$	19	100	5.3
7	2-F Ph	40	19	0.5
8	3-F Ph	29	18	0.6
9	4-F Ph	27	29	1.1
10	3-MeO Ph	110	88	0.8
11	4-MeO Ph	40	104	2.6
12	3-CN Ph	144	304	2.1
13	4-CN Ph	70	308	4.4
14	Ph	50	33	0.7
15	2-furanyl	20	47	2.4
16	2-thiophenyl	13	18	1.4
17	-	438	851	1.9
I		13	0.2	0.02
Etomidate		0.5	0.1	0.5
Metyrapone		15	72	4.8

[a] Hamster fibroblasts expressing human CYP11B1; substrate deoxycorticosterone, 100 nM. [b] Mean value of at least three experiments, relative standard deviation usually less than 25%. [c] Hamster fibroblasts expressing human CYP11B2; substrate deoxycorticosterone, 100 nM. [d] IC_{50} CYP11B2/IC_{50} CYP11B1.

corresponding *meta*- substituted compounds **10** and **12** (IC_{50} = 110 and 144 nM). Interestingly, the *para*-substituted analogues **11** and **13** (SF = 2.6 and 4.4) also exhibited a better selectivity compared to the non- or *meta*- substituted compounds.

Furthermore, replacement of the phenyl group by its less bulky bioisosters 2-furanyl (**15**) and 2-thiophenyl (**16**) elevated the inhibition of CYP11B1 to around 15 nM. However, analogue **17** with a methyl and an additional imidazolyl group at the CH bridge exhibited a dramatic decrease in inhibitory activity (IC_{50} = 438 nM). It is interesting to observe that this exchange of phenyl by heterocycles (**15**–**17**) actually improved the

selectivity compared to **14**.

With compound **4** as the most selective one (SF = 11), the synthesized compounds are much more selective than their parent compounds, especially compared to reference compound **I** (SF = 0.02). This achievement is certainly attributed to the introduction of the CH bridge between the tetrahydropyrroloquinolinone core and the N-containing heterocycle, and probably also to the replacement of isoquinolinyl by imidazolyl.

No Significant Difference between *R*- and *S*-Enantiomers in Biological Activity. In order to investigate the possible influence of different configurations on inhibitory potency or selectivity, the racemic compounds **4** and **7** were separated by HPLC and tested. However, no significant difference between the bioactivity of the corresponding pure enantiomers was found for either compound (data not shown).

Selectivity against Human CYP17 and CYP19. Since CYP17 and CYP19 are crucial enzymes involved in androgen and estrogen biosynthesis respectively, the selectivities for four of the most potent and / or selective compounds against these two enzymes were further tested as criteria to evaluate safety (Table 2). As can be seen, compounds **3** and **4** exhibited weak CYP17 inhibition with IC_{50} values over 1500 nM, whereas compounds **6** and **12** showed almost no effect (IC_{50} > 5000 nM). Moreover, due to the structural similarity of the synthesized compounds to the clinically used CYP19 inhibitors like letrozole, the inhibition of CYP19 was not a surprise. Compounds **6** and **12** showed strong inhibition with IC_{50} values of 17 nM and 43 nM (Table 2), respectively, which is probably due to the presence of methylene or phenyl substituents at the CH bridge. In contrast, compounds **3** and **4** with saturated alkyl groups exhibited only modest inhibition of CYP19 (IC_{50} > 200 nM, Table 2). Accordingly, good selectivity was achieved with selectivity factor ($IC_{50\ CYP19}$ / $IC_{50\ CYP11B1}$) of 357 for compound **3** and 104 for compound **4** due to the extremely potent inhibition of CYP11B1.

Table 2. Inhibition of CYP17, CYP19 and rat CYP11B1 by selected compounds.

Compd	CYP17[a] IC_{50} [e] (nM)	CYP19[b] IC_{50} [e] (nM)	Rat CYP11B1[c] Inhib. %[f]
3	1550	>500	n.d.
4	2880	228	91
6	>5000	17	n.d.
11	>5000	43	n.d.
I			31

[a] *E. coli* expressinghuman CYP17; substrate progesterone, 25 μM; Abiraterone, IC_{50} = 72 nM. [b] Human placental CYP19; substrate androstenedione, 500 nM, inhibitor concentration 500 nM; fadrozole, IC_{50} = 41 nM. [c] Hamster fibroblasts expressing rat CYP11B1; substrate deoxycorticosterone, 500 nM; inhibitor concentration 2.0 μM. [d] IC_{50} CYP19 / IC_{50} CYP11B2. [e] Mean value of at least three experiments, standard deviation usually less than 10%; n.d. = not determined.[f] Mean value of at least three experiments, relative standard deviation usually less than 20%.

Inhibition of Rat CYP11B1. Drug candidates always need to be evaluated in animals (usually rats) for pharmacokinetic and pharmacodynamic properties before finally entering into clinical trials. Potent inhibition of rat enzyme is therefore the precondition to observe the desired curative effects in the rat disease

models. Compound **4** showed 91% inhibition of rat CYP11B1 at a concentration of 2 μM, in comparison to 31% for reference compound **I**. This high potency toward rat enzyme facilitates further evaluation in vivo.

Conclusion

Starting from two leads, etomidate and reference compound, which both showed strong inhibition of CYP11B1 but even more potent inhibition of CYP11B2, novel imidazol-1-ylmethyl substituted 1,2,5,6-tetrahydro-pyrrolo[3,2,1-*ij*]quinolin-4-ones were designed and synthesized aiming at the development of potent and selective CYP11B1 inhibitors for the treatment of Cushing's syndrome. The replacement of the phenyl moiety in the ETO scaffold by a tetrahydropyrroloquinolinone group from reference compound **I** sustained the potent inhibition of CYP11B1, while the improved selectivity was attributed to the CH bridge between the tetrahydropyrroloquinolinone core and the heme binding imidazolyl.

It has been elucidated that the bulkiness of the substituents on the CH bridge was a determinant of inhibitory potency. With increasing bulkiness of the aliphatic chains, the inhibitory potency increased from about 20 nM to less than 5 nM. However, when phenyl was introduced, the inhibition was decreased to 50 nM. The inhibitory activity was improved again to around 15 nM when furanyl and thiophenyl were employed as less bulky bioisosters of phenyl. Moreover, similar steric limitations were observed when substituents were introduced on phenyl group. Intriguingly, compounds with substitutents at the *para*-position of phenyl group showed improved inhibition and selectivity compared to non- or *meta*- substituted phenyl analogues.

Furthermore, compound **4** was identified as a CYP11B1 inhibitor more potent (IC_{50} = 2.2 nM) and selective (SF = 11) than the lead compounds and metyrapone, the drug in clinical use. Since this compound also showed potent inhibition of rat CYP11B1 (facilitating in vivo test) and good selectivity over CYP17 and CYP19, it is considered to be a promising lead compound for further evaluation in vivo.

Experimental Section

Biology

Inhibition of CYP11B1 and CYP11B2

V79MZh cells expressing human/rat CYP11B1 or CYP11B2 were incubated with [1,2-^{3}H]-11-deoxycorticosterone as the substrate and the inhibitor at different concentrations.. The assay was performed as previously described.[15]

CYP17 preparation and assay

Human CYP17 was expressed in *E. coli* [17a] (coexpressing human CYP17 and NADPH-P450 reductase), and the assay was performed using method previously described, progesterone as the substrate and NADPH as the cofactor.[17b]

CYP19 preparation and assay

Human CYP19 was obtained from microsomal preparations of human placenta and the assay was

performed using the ^3H_2O-method as previously described and [1β-^{3}H]androstenedione as the substrate.[18]

Chemistry

General Method

Melting points were determined on a Mettler FP1 melting point apparatus and are uncorrected. ^{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. All coupling constants (*J*) are given in Hz. ESI (electrospray ionization) mass spectra were determined on a TSQ quantum (Thermo Electron Corporation) instrument. The purities of the final compounds were controlled by Surveyor-LC-system. Purities were greater than 95%. 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). Reagents and solvents commercially available were used directly without further purification.

Method A: Friedel-Crafts Acylation

To a solution of **1c** (1.0 equiv) and $AlCl_3$ (3.0–7.0 equiv) in dry dichloromethane was added dropwise an acyl chloride (1.5–2.0 equiv) at room temperature. The mixture was then refluxed for 18 h, cooled to ambient temperature and quenched by addition of a cold mixture of concentrated hydrochloric acid (2.0 equiv) and water. Extraction with EtOAc three times, followed by drying over $MgSO_4$ and removal of the solvent in vacuo gave a ketone.

Method B: Borohydride Reduction

The ketone (1.0 equiv) was dissolved in methanol. Sodium hydride (1.2–2.0 equiv) was added in portions at 0 °C. After stirring for 1 h at the same temperature, water was added and the resulting mixture was extracted with EtOAc three times. The combined organic layers were dried over $MgSO_4$ and concentrated in vacuo to give the corresponding alcohol.

Method C: Imidazylation

A solution of the obtained alcohol (1.0 equiv) in dry THF was added to a solution of thionylbis(imidazo1e) (prepared previously by reaction of imidazole (16 equiv) with thionyl chloride (4.0 equiv) in dry THF and filtration to remove precipitated imidazole hydrochloride) at 0 °C. The reaction mixture was stirred for 1 h at 0 °C and an additional 18–30 h at ambient temperature. Water was added and the mixture extracted with ethyl acetate three times, the combined organic extracts washed with water and brine, and the solvent was evaporated in vacuo after drying over $MgSO_4$. The crude product was purified by flash chromatography to yield the corresponding product.

Method D: Stille Coupling

3c (1.0 equiv), dichlorobis(triphenylphosphine)palladium(II) (0.1 equiv) and an acyl chloride (2.0 equiv) were suspended in 25 mL dry toluene under an atmosphere of nitrogen. The mixture was refluxed for 2 h

before it was concentrated in vacuo. The resulting residue was purified by flash chromatography on silica gel to yield the corresponding ketone.

8-[1-(1*H*-imidazol-1-yl)-2-methylpropyl]-1,2,5,6-tetrahydropyrrolo[3,2,1-*ij*]quinolin-4-one (3). The title compound was synthesized according to Method C using **3a** (0.20 g, 0.82 mmol), imidazole (0.90 g, 13.0 mmol), $SOCl_2$ (0.24 mL, 3.26 mmol) and dry THF (10 mL). The crude product was purified by flash chromatography (MeOH/CH_2Cl_2, 0 to 1:50) to yield pale yellow solids (35 mg, 15%). mp 150–152 °C, R_f = 0.20 (MeOH/CH_2Cl_2, 1:20). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 0.91 (t, J = 6.9 Hz, 6H), 2.54 (m, 1H), 2.66 (t, J = 7.8 Hz, 2H), 2.94 (t, J = 7.8 Hz, 2H), 3.16 (t, J = 8.5 Hz, 2H), 4.07 (t, J = 8.5 Hz, 2H), 4.55 (d, J = 10.5 Hz, 1H), 6.92 (s, 1H), 6.99 (s, 1H), 7.01 (s, 1H), 7.06 (s, 1H), 7.68 (s, 1H). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 20.1, 20.5, 24.4, 27.7, 31.4, 32.5, 45.4, 69.5, 117.4, 120.4, 122.3, 124.7, 128.9, 129.6, 134.6, 136.1, 141.5, 167.5. MS (ESI) m/z = 229 [M-imidazole]$^+$.

Resolution of Racemate. Enantiomers were separated via preparative HPLC on a Agilent Technologies 1200 series system (quaternary pump, MWD, fraction collector) using a NucleoCel Delta column, 0.8 cm × 25 cm. Retention times (t_R) were determined with an analytical column using 0.3 mL/min and UV detection (254 nm). The ee values were measured on a Merck-Hitachi LaChrome D-7000 System (isocratic pump L7100, diode array detector L7455, autosampler L-7200) using a Chiracel OD-H column, 0.46 cm × 25 cm.

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Supporting Information

1. The synthetic procedures and characterization of all intermediates and all final compounds as well as ^{1}H-NMR and ^{13}C-NMR of all final compounds except for compound 3.

Method A: Friedel-Crafts Acylation

To a solution of **1c** (1.0 equiv) and $AlCl_3$ (3.0–7.0 equiv) in dry dichloromethane was added dropwise an acyl chloride (1.5–2.0 equiv) at room temperature. The mixture was then refluxed for 18 h, cooled to ambient temperature and quenched by addition of a cold mixture of concentrated hydrochloric acid (2.0 equiv) and water. Extraction with EtOAc three times, followed by drying over $MgSO_4$ and removal of the solvent in vacuo gave a ketone.

Method B: Borohydride Reduction

The ketone (1.0 equiv) was dissolved in methanol. Sodium hydride (1.2–2.0 equiv) was added in portions at 0 °C. After stirring for 1 h at the same temperature, water was added and the resulting mixture was extracted with EtOAc three times. The combined organic layers were dried over $MgSO_4$ and concentrated in vacuo to give the corresponding alcohol.

Method C: Imidazylation

A solution of the obtained alcohol (1.0 equiv) in dry THF was added to a solution of thionylbis(imidazo1e) (prepared previously by reaction of imidazole (16 equiv) with thionyl chloride (4.0 equiv) in dry THF and filtration to remove precipitated imidazole hydrochloride) at 0 °C. The reaction mixture was stirred for 1 h at 0 °C and an additional 18–30 h at ambient temperature. Water was added and the mixture extracted with EtOAc three times, the combined organic extracts washed with water and brine, and the solvent was evaporated in vacuo after drying over $MgSO_4$. The crude product was purified by flash chromatography to yield the corresponding product.

Method D: Stille Coupling

3c (1.0 equiv), dichlorobis(triphenylphosphine)palladium(II) (0.1 equiv) and an acyl chloride (2.0 equiv) were suspended in 25 mL dry toluene under an atmosphere of nitrogen. The mixture was refluxed for 2 h

before it was concentrated in vacuo. The resulting residue was purified by flash chromatography on silica gel to yield the corresponding ketone.

3-Chloro-1-(2,3-dihydro-indol-1-yl)-propan-1-one (1d). To a solution of indoline (25.0 mL, 223 mmol) and pyridine in 100 ml THF was added dropwise 3-chloropropanoyl chloride (23.4 ml, 245 mmol) at 0 °C under N_2. Immediately a lot of precipitate was formed. The suspension was allowed to warm to ambient temperature and stirred overnight. TLC monitored the reaction complete. Water was added to quench followed by extraction with EtOAc (3 x 100 ml). After the combined organic layers were washed with brine (3 x 200 ml) and dried over $MgSO_4$. Evaporation gave pink solids, which were directly used in the next step.

1,2,5,6-tetrahydropyrrolo[3,2,1-*ij*]quinolin-4-one (1c). A molten mixture of **1d** (44.5 g, 212 mmol) and $AlCl_3$ (149 g, 1.12 mol) was stirred at 140 °C for 4 h. Upon cooling to 0 °C, a mixture of water/ice (500 g) was added to decompose excess $AlCl_3$. The resulting solution was extracted with EtOAc (3 x 200 ml), followed by drying over $MgSO_4$ and removal of the solvent gave a yellow solid, which was purified by flash chromatography (EtOAc/n-Hexane, 1:20 to 2:5) to yield **1c** as white solids (25.7 g, 70%). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 2.68 (t, J = 7.8 Hz, 2H), 2.97 (t, J = 7.8 Hz, 2H), 3.18 (t, J = 8.5 Hz, 2H), 4.07 (t, J = 8.5 Hz, 2H), 6.92 (t, J = 7.4 Hz, 1H), 6.99 (d, J = 7.5 Hz, 1H); 7.07 (d, J = 7.3 Hz, 1H); ^{13}C-NMR (125 MHz, $CDCl_3$): δ_C 24.4, 27.8, 31.6, 45.1, 120.2, 123.2, 123.3, 125.3, 128.9, 141.3, 167.7; MS (ESI) *m/z* = 174 [M^++H].

8-Bromo-1,2,5,6-tetrahydro-pyrrolo[3,2,1-*ij*]quinolin-4-one (3d). To a solution of **1c** (1.00 g, 5.77 mmol) in 18 ml dry DMF was added dropwise a solution of *N*-bromosuccinimide (1.09 g, 6.12 mmol) in 18 ml dry DMF at 0 °C. The mixture was stirred at 0 °C for 2 h, then 36 ml water was added and the resulting solution was extracted with EtOAc (3 x 40 ml). The organic phase was washed with water (2 x 20 ml), then dried over $MgSO_4$ and evaporated, affording brown solids which was purified by washing with flash chromatography (EtOAc/n-Hexane, 2:5) to yield **3d** as white solids (0.93 g, 65%). MS (ESI) *m/z* = 252 $[M]^+$.

8-(Tributylstannanyl)-1,2,5,6-tetrahydropyrrolo[3,2,1-*ij*]quinolin-4-one (3c). To a solution of **3d** (9.07 g, 36.0 mmol) and 1,1,1,2,2,2-hexabutyldistannane (29.1 mL, 57.6 mmol) in 40 mL degassed dry toluene was added tetrakis(triphenylphosphine)palladium(0) (4.16g, 3.60 mmol) under an atmosphere of nitrogen, and the mixture was stirred overnight at 90 °C. After cooling to ambient temperature, the mixture was concentrated in vacuo. The crude product was purified by flash chromatography (EtOAc/n-Hexane, 1:10, R_f = 0.1) to yield

pure **3c** as a light yellow oil (7.50 g, 45%). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 0.89 (t, *J* = 7.3 Hz, 9H), 1.03 (m, 6H), 1.34 (sext, *J* = 7.3 Hz, 6H), 1.53 (m, 6H), 2.68 (t, *J* = 7.8 Hz, 2H), 2.97 (t, *J* = 7.8 Hz, 2H), 3.18 (t, *J* = 8.4 Hz, 2H), 4.05 (t, *J* = 8.4 Hz, 1H), 7.05 (s, 1H), 7.16 (s, 1H); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 9.7, 13.6, 24.4, 27.4, 27.8, 29.1, 31.8, 45.0, 120.0, 128.8, 130.9, 132.9, 135.9, 141.5, 167.7.

8-Acetyl-1,2,5,6-tetrahydro-pyrrolo[3,2,1-*ij*]quinolin-4-one (1b). The title compound was synthesized according to Method A using **1c** (1.79 g, 10.0 mmol), $AlCl_3$ (9.31 g, 70.0 mmol) and acetyl chloride (1.18 g, 15.0 mmol) in dry CH_2Cl_2 (50 mL) to yield the crude product as a yellow solid (1.79 g, 83%). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 2.56 (s, 3H), 2.72 (t, *J* = 7.8 Hz, 2H), 3.02 (t, *J* = 7.8 Hz, 2H), 3.23 (t, *J* = 8.5 Hz, 2H), 4.13 (t, *J* = 8.5 Hz, 2H), 7.67 (s, 1H), 7.72 (s, 1H). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 24.2, 26.5, 27.3, 31.4, 45.7, 119.6, 124.2, 127.0, 129.2, 133.2, 145.7, 167.9, 196.8.

8-(1-Hydroxy-ethyl)-1,2,5,6-tetrahydro-pyrrolo[3,2,1-*ij*]quinolin-4-one (1a). The title compound was synthesized according to Method B using **1b** (1.79 g, 8.31 mmol) and sodium hydride (452 mg, 12.0 mmol) in Methanol (50 mL) to yield a yellow solid (1.81 g, 100%). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 1.48 (d, *J* = 6.4 Hz, 3H), 2.01 (s, br, 1H) , 2.66 (t, *J* = 7.8 Hz, 2H), 2.95 (t, *J* = 7.8 Hz, 2H), 3.16 (t, *J* = 8.4 Hz, 2H), 4.06 (t, *J* = 8.4 Hz, 2H), 4.84 (q, *J* = 6.2 Hz, 1H), 7.02 (s, 1H), 7.10 (s, 1H). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 24.4, 25.4, 27.7, 31.6, 45.4, 70.3, 120.0, 120.6, 122.8, 129,1, 140.7, 141.5, 167.6.

8-Propionyl-1,2,5,6-tetrahydro-pyrrolo[3,2,1-ij]quinolin-4-one (2b). The title compound was synthesized according to Method A using **1c** (1.79 g, 10.0 mmol), $AlCl_3$ (4.00 g, 30 mmol) and propionyl chloride (1.31 ml, 15.0 mmol) in dry dichloromethane (80 mL) to yield the crude product, which was purified by flash chromatography on silica gel (hexanes/ EtOAc, 3:7, R_f = 0.16) to yield a green solid (1.20 g, 52%). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 1.20 (t, *J* = 7.2, 3H), 2.70 (t, *J* = 7.6, 2H), 2.93 (t, *J* = 7.2, 2H), 3.00 (t, *J* = 7.6, 2H), 3.21 (t, *J* = 8.5, 2H), 4.12 (t, *J* = 8.5, 2H), 7.71 (1H, s), 7.67 (1H, s). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 8.5, 24.25, 27.3, 31.4, 31.6, 45.7, 119.6, 123.8, 126.5, 129.1, 132.9, 145.4, 167.84, 199.5.

8-(1-Hydroxy-propyl)-1,2,5,6-tetrahydro-pyrrolo[3,2,1-ij]quinolin-4-one (2a). The title compound was synthesized according to Method B using **2b** (1.20 g, 5.20 mmol) and sodium hydride (236 mg, 6.21 mmol) in Methanol (26 mL) to yield a yellow solid (0.63 g, 52%). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 0.90 (t, *J* = 7.2, 3H), 1.68–1.83 (m, 2H), 2.66 (t, *J* = 7.9, 2H), 2.94 (t, *J* = 7.9, 2H), 3.16 (t, *J* = 8.5, 2H), 4.06 (t, *J* = 8.5, 2H),

4.52 (t, *J* = 6.6, 1H), 6.97 (s, 1H), 7.05 (s, 1H),. ^{13}C-NMR (125 MHz, $CDCl_3$): δ 10.5, 24.8, 28.0, 31.9, 32.3, 45.6, 76.3, 120.2, 121.4, 123.6, 129.2, 140.5, 141.0, 167.8.

8-Isobutyryl-1,2,5,6-tetrahydro-pyrrolo[3,2,1-*ij*]quinolin-4-one (3b). The title compound was synthesized according to Method D using **3c** (1.50 g, 3.24 mmol), isobutyryl chloride (0.68 mL, 6.49 mmol) and dichlorobis(triphenyl phosphine)palladium(II) (0.23 g, 0.32 mmol) in dry toluene (20 mL) to yield the crude product, which was purified by flash chromatography on silica gel (EtOAc/PE, 1:50 to 2:5) to yield a yellow solid (0.19 g, 24%). R_f = 0.15 (EtOAc/PE, 1:1). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 1.21 (d, *J* = 6.8 Hz, 6H), 2.72 (t, *J* = 7.8 Hz, 2H), 3.02 (t, *J* = 7.8 Hz, 2H), 3.23 (t, *J* = 8.5 Hz, 2H), 3.50 (sept, *J* = 6.8 Hz, 1H), 4.13 (t, *J* = 8.5 Hz, 2H), 7.68 (s, 1H), 7.72 (s, 1H).

8-(1-Hydroxy-2-methyl-propyl)-1,2,5,6-tetrahydro-pyrrolo[3,2,1-*ij*]quinolin-4-one (3a). The title compound was synthesized according to Method B using **3b** (1.88 g, 0.77 mmol) and sodium hydride (59.0 mg, 1.54 mmol) in Methanol (8 mL) to yield a yellow solid (0.19 g, 100%). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 0.79 (d, *J* = 6.8 Hz, 3H), 1.02 (d, *J* = 6.6 Hz, 3H), 1.69 (s, br, 1H), 1.92 (sext, *J* = 6.8 Hz, 1H), 2.67 (t, *J* = 7.8 Hz, 2H), 2.96 (t, *J* = 7.8 Hz, 2H), 3.17 (t, *J* = 8.4 Hz, 2H), 4.08 (t, *J* = 8.4 Hz, 2H), 4.28 (d, *J* = 7.2 Hz, 1H), 6.95 (s, 1H), 7.03 (s, 1H). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 18.4, 19.1, 24.5, 27.7, 31.6, 35.4, 45.4, 80.2, 119.7, 121.6, 123.9, 128.8, 139.3, 140.7, 167.6.

8-Cyclopropanecarbonyl-1,2,5,6-tetrahydro-pyrrolo[3,2,1-*ij*]quinolin-4-one (4b). The title compound was synthesized according to Method D using **3c** (1.50 g, 3.24 mmol), cyclopropane carbonyl chloride (0.59 mL, 6.49 mmol) and dichlorobis(triphenyl phosphine)palladium(II) (0.23 g, 0.32 mmol) in dry toluene (25 mL) to yield the crude product, which was purified by flash chromatography on silica gel (EtOAc/PE, 1:50 to 1:1) to yield a pale yellow solid (0.58 g, 74%). R_f = 0.07 (EtOAc/PE, 1:1). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 1.01 (m, 2H), 1.21 (m, 2H), 2.60 (m, 1H), 2.72 (t, *J* = 7.8 Hz, 2H), 3.03 (t, *J* = 7.8 Hz, 2H), 3.23 (t, *J* = 8.5 Hz, 2H), 4.14 (t, *J* = 8.5 Hz, 2H), 7.74 (s, 1H), 7.78 (s, 1H).

8-(Cyclopropyl-hydroxy-methyl)-1,2,5,6-tetrahydro-pyrrolo[3,2,1-*ij*]quinolin-4-one (4a). The title compound was synthesized according to Method B using **4b** (0.58 g, 0.24 mmol) and sodium hydride (0.18 g, 4.80 mmol) in Methanol (15 mL) to yield a yellow solid (0.56 g, 96%). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 0.33–0.38 (m, 1H), 0.45–0.50 (m, 1H), 0.54–0.59 (m, 1H), 0.63–0.68 (m, 1H), 1.17–1.24 (m, 1H), 1.93 (d, *J* = 2.9 Hz, 1H), 2.68 (t, *J* = 7.8 Hz, 2H), 2.97 (t, *J* = 7.8 Hz, 2H), 3.18 (t, *J* = 8.4 Hz, 2H), 3.93 (dd, *J* = 2.7, 8.4 Hz, 1H), 4.08 (t, *J* = 8.4 Hz, 2H), 7.07 (s, 1H), 7.15 (s, 1H).

8-Cyclobutanecarbonyl-1,2,5,6-tetrahydro-pyrrolo[3,2,1-*ij*]quinolin-4-one (5b). The title compound was synthesized according to Method D using **3c** (1.20 g, 2.60 mmol), cyclobutane carbonyl chloride (0.42 mL, 5.20 mmol) and dichlorobis(triphenyl phosphine)palladium(II) (0.18 g, 0.26 mmol) dry toluene (20 mL) to yield the crude product, which was purified by flash chromatography on silica gel (EtOAc/PE, 1:50 to 1:2) to yield a pale yellow solid (0.42 g, 64%). R_f = 0.09 (EtOAc/PE, 1:1). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 1.87–1.94 (m, 1H), 2.03–2.12 (m, 1H), 2.24–2.31 (m, 2H), 2.36–2.44 (m, 2H), 2.71 (t, *J* = 7.8 Hz, 2H), 3.01 (t, *J* = 7.8 Hz, 2H), 3.21 (t, *J* = 8.5 Hz, 2H), 3.93 (quin, *J* = 8.5 Hz, 1H), 4.12 (t, *J* = 8.5 Hz, 2H), 7.60 (s, 1H), 7.65 (s, 1H).

8-(Cyclobutyl-hydroxy-methyl)-1,2,5,6-tetrahydro-pyrrolo[3,2,1-*ij*]quinolin-4-one (5a). The title compound was synthesized according to Method B using **5b** (0.58 g, 0.24 mmol) and sodium hydride (0.18 g, 4.80 mmol) in Methanol (15 mL) to yield a yellow solid (0.56 g, 96%) after purification by flash chromatography on silica gel ($MeOH/CH_2Cl_2$, 0 to 1:100). R_f = 0.15 ($MeOH/CH_2Cl_2$, 1:20). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 1.73–1.90 (m, 4H), 1.92 (d, *J* = 3.2 Hz, 1H), 1.95–2.02 (m, 1H), 2.07–2.14 (m, 1H), 2.60 (quad, *J* = 8.0 Hz, 1H), 2.65 (t, *J* = 7.8 Hz, 2H), 2.94 (t, *J* = 7.8 Hz, 2H), 3.16 (t, *J* = 8.4 Hz, 2H), 4.06 (t, *J* = 8.4 Hz, 2H), 4.50 (dd, *J* = 3.3, 8.2 Hz, 1H), 6.95 (s, 1H), 7.03 (s, 1H). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 17.7, 24.4, 25.0, 27.7, 31.6, 42.5, 45.3, 78.6, 119.6, 121.3, 123.6, 128.9, 138.7, 140.8, 167.6.

8-(2-Fluoro-benzoyl)-1,2,5,6-tetrahydro-pyrrolo[3,2,1-*ij*]quinolin-4-one (7b). The title compound was synthesized according to Method D using **3c** (1.20 g, 2.60 mmol), 2-fluorobenzoyl chloride (0.42 mL, 5.20 mmol) and dichlorobis(triphenyl phosphine)palladium(II) (0.18 g, 0.26 mmol) dry toluene (20 mL) to yield the crude product, which was purified by flash chromatography on silica gel (EtOAc/PE, 1:20 to 1:2) to yield a yellow semisolid (0.64 g, 84%). R_f = 0.15 (EtOAc/PE, 1:1). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 2.72 (t, *J* = 7.8 Hz, 2H), 3.02 (t, *J* = 7.8 Hz, 2H), 3.24 (t, *J* = 8.4 Hz, 2H), 4.13 (t, *J* = 8.4 Hz, 2H), 7.11–7.20 (m, 3H), 7.26–7.31 (m, 2H), 7.38 (td, *J* = 1.8, 7.8 Hz, 1H).

8-[(2-Fluoro-phenyl)-hydroxy-methyl]-1,2,5,6-tetrahydro-pyrrolo[3,2,1-*ij*]quinolin-4-one (7a). The title compound was synthesized according to Method B using **7b** (0.64 g, 2.18 mmol) and sodium hydride (0.17 g, 4.36 mmol) in Methanol (20 mL) to yield a yellow solid (0.17 g, 26%) after purification by flash chromatography on silica gel ($MeOH/CH_2Cl_2$, 1:500 to 1:100). R_f = 0.21 ($MeOH/CH_2Cl_2$, 1:20). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 2.28 (d, *J* = 2.9 Hz, 1H), 2.65 (t, *J* = 7.8 Hz, 2H), 2.93 (t, *J* = 7.8 Hz, 2H), 3.15 (t, *J* =

8.5 Hz, 2H), 4.06 (t, *J* = 8.5 Hz, 2H), 6.08 (d, *J* = 2.8 Hz, 1H), 7.02 (m, 1H), 7.04 (s, 1H), 7.11 (s, 1H), 7.18 (td, *J* = 1.1, 7.5 Hz, 1H), 7.27 (m, 1H), 7.57 (td, *J* = 1.7, 7.6 Hz, 1H).

8-(3-Fluoro-benzoyl)-1,2,5,6-tetrahydro-pyrrolo[3,2,1-*ij*]quinolin-4-one (8b). The title compound was synthesized according to Method D using **3c** (1.50 g, 3.24 mmol), 3-fluorobenzoyl chloride (0.79 mL, 6.48 mmol) and dichlorobis(triphenyl phosphine)palladium(II) (0.23 g, 0.32 mmol) dry toluene (20 mL) to yield the crude product, which was purified by flash chromatography on silica gel (EtOAc/PE, 1:50 to 1:2) to yield a yellow solid (0.70 g, 73%). R_f = 0.15 (EtOAc/PE, 1:1). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 2.74 (t, *J* = 7.8 Hz, 2H), 3.03 (t, *J* = 7.8 Hz, 2H), 3.24 (t, *J* = 8.5 Hz, 2H), 4.15 (t, *J* = 8.5 Hz, 2H), 7.25–7.29 (m, 1H), 7.42–7.48 (m, 2H), 7.52 (m, 2H), 7.57 (s, 1H).

8-[(3-Fluoro-phenyl)-hydroxy-methyl]-1,2,5,6-tetrahydro-pyrrolo[3,2,1-*ij*]quinolin-4-one (8a). The title compound was synthesized according to Method B using **8b** (0.49 g, 1.66 mmol) and sodium hydride (0.13 g, 3.33 mmol) in Methanol (15 mL) to yield a yellow solid (0.43 g, 87%) after purification by flash chromatography on silica gel (EtOAc/PE, 1:500 to 1:100). R_f = 0.06 (EtOAc/PE, 1:1). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 2.28 (d, *J* = 2.9 Hz, 1H), 2.63 (t, *J* = 7.8 Hz, 2H), 2.92 (t, *J* = 7.8 Hz, 2H), 3.14 (t, *J* = 8.5 Hz, 2H), 4.04 (t, *J* = 8.5 Hz, 2H), 5.76 (s, 1H), 6.94 (m, 1H), 6.99 (s, 1H), 7.06 (s, 1H), 7.13 (m, 2H), 7.29 (m, 1H). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 24.4, 27.7, 31.5, 45.4, 75.5 (d, $^4J_{C,F}$ = 1.3 Hz), 113.2 (d, $^2J_{C,F}$ = 22.1 Hz), 114.2 (d, $^2J_{C,F}$ = 21.1 Hz), 120.1, 121.8, 124.1, 129.3, 130.0 (d, $^3J_{C,F}$ = 8.2 Hz), 139.2, 141.0, 146.7 (d, $^3J_{C,F}$ = 6.5 Hz), 163.0 (d, $^1J_{C,F}$ = 246 Hz), 167.6.

8-(4-Fluoro-benzoyl)-1,2,5,6-tetrahydro-pyrrolo[3,2,1-*ij*]quinolin-4-one (9b). The title compound was synthesized according to Method D using **3c** (3.11 g, 6.73 mmol), 4-fluorobenzoyl chloride (1.60 mL, 13.5 mmol) and dichlorobis(triphenyl phosphine)palladium(II) (0.47 g, 0.67 mmol) dry toluene (30 mL) to yield the crude product, which was purified by flash chromatography on silica gel (EtOAc/PE, 7:3) to yield a yellow solid (1.29 g, 65%). R_f = 0.15 (EtOAc/PE, 1:1). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 2.73 (t, *J* = 7.8 Hz, 2H), 3.02 (t, *J* = 7.8 Hz, 2H), 3.23 (t, *J* = 8.5 Hz, 2H), 4.15 (t, *J* = 8.5 Hz, 2H), 7.16 (m, 2H), 7.50 (s, 1H), 7.54 (s, 1H), 7.78 (m, 2H).

8-[(4-Fluoro-phenyl)-hydroxy-methyl]-1,2,5,6-tetrahydro-pyrrolo[3,2,1-*ij*]quinolin-4-one (9a). The title compound was synthesized according to Method B using **9b** (0.55 g, 1.86 mmol) and sodium hydride (0.14 g, 3.71 mmol) in Methanol (10 mL) to yield a pale yellow solid (0.37 g, 66%) after purification by flash chromatography on silica gel ($MeOH/CH_2Cl_2$, 1:500 to 1:100). R_f = 0.04 ($MeOH/CH_2Cl_2$, 1:50). ^{1}H-NMR

(500 MHz, $CDCl_3$): δ 2.32 (d, J = 3.4 Hz, 1H), 2.65 (t, J = 7.8 Hz, 2H), 2.93 (t, J = 7.8 Hz, 2H), 3.14 (t, J = 8.5 Hz, 2H), 4.06 (t, J = 8.5 Hz, 2H), 5.77 (d, J = 3.1 Hz, 1H), 6.99 (s, 1H), 7.03 (m, 2H), 7.06 (s, 1H), 7.35 (m, 2H). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 24.4, 27.7, 31.5, 45.4, 75.5, 115.3, 120.1, 121.7, 124.0, 128.0 (d, $^3J_{C,F}$ = 8.1 Hz), 128.5 (d, $^2J_{C,F}$ = 12.2 Hz), 129.2, 132.1 (d, $^3J_{C,F}$ = 9.9 Hz), 139.4, 139.8 (d, $^4J_{C,F}$ = 3.0 Hz), 141.0, 161.1 (d, $^1J_{C,F}$ = 246 Hz), 167.6. MS (ESI) *m/z* = 298 $[M+H]^+$.

8-(3-Methoxy-benzoyl)-1,2,5,6-tetrahydro-pyrrolo[3,2,1-*ij*]quinolin-4-one (10b). The title compound was synthesized according to Method D using **3c** (1.50 g, 3.24 mmol), m-anisoyl chloride (0.98 mL, 6.48 mmol) and dichlorobis(triphenyl phosphine)palladium(II) (0.23 g, 0.32 mmol) dry toluene (20 mL) to give the crude product, which was purified by flash chromatography on silica gel (EtOAc/PE, 1:20 to 4:5) to yield a pale yellow solid (0.70 g, 69%). R_f = 0.13 (EtOAc/PE, 1:1). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 2.73 (t, J = 7.7 Hz, 2H), 3.02 (t, J = 7.7 Hz, 2H), 3.23 (t, J = 8.5 Hz, 2H), 3.86 (s, 3H), 4.15 (t, J = 8.5 Hz, 2H), 7.12 (m, 1H), 7.28 (m, 2H), 7.38 (t, J = 7.9 Hz, 1H), 7.54 (s, 1H), 7.59 (s, 1H). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 24.2, 27.4, 31.5, 45.7, 55.5, 114.3, 118.2, 119.5, 122.3, 126.1, 128.8, 129.0, 129.2, 133.1, 139.7, 145.4, 159.6, 167.9, 195.5.

8-[Hydroxy-(3-methoxy-phenyl)-methyl]-1,2,5,6-tetrahydro-pyrrolo[3,2,1-*ij*]quinolin-4-one (10a). The title compound was synthesized according to Method B using **10b** (0.69 g, 2.24 mmol) and sodium hydride (0.17 g, 4.48 mmol) in Methanol (10 mL) to yield a white solid (0.57 g, 82%) after purification by flash chromatography on silica gel (MeOH/CH_2Cl_2, 0 to 1:50). R_f = 0.25 (MeOH/CH_2Cl_2, 1:20). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 2.31 (d, J = 3.4 Hz, 1H), 2.64 (t, J = 7.8 Hz, 2H), 2.93 (t, J = 7.8 Hz, 2H), 3.14 (t, J = 8.5 Hz, 2H), 3.80 (s, 3H), 4.05 (t, J = 8.5 Hz, 2H), 5.75 (d, J = 3.2 Hz, 1H), 6.81 (ddd, J = 0.8, 2.5, 8.2 Hz, 1H), 6.96 (m, 2H), 7.02 (s, 1H), 7.08 (s, 1H), 7.26 (m, 1H). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 24.4, 27.7, 31.6, 45.4, 55.2, 76.1, 112.0, 112.7, 118.6, 120.0, 121.8, 124.0, 129.1, 129.6, 139.4, 140.9, 145.7. 159.8, 167.6.

8-(4-Methoxy-benzoyl)-1,2,5,6-tetrahydro-pyrrolo[3,2,1-*ij*]quinolin-4-one (11b). The title compound was synthesized according to Method D using **3c** (2.00 g, 4.33 mmol), p-anisoyl chloride (1.17 mL, 8.66 mmol) and dichlorobis(triphenyl phosphine)palladium(II) (0.30 g, 0.43 mmol) dry toluene (20 mL) to yield the crude product, which was purified by flash chromatography on silica gel (EtOAc/PE, 1:50 to 4:5) to yield a pale yellow solid (0.85 g, 64%). R_f = 0.30 (EtOAc/PE, 1:1). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 2.73 (t, J = 7.8 Hz, 2H), 3.02 (t, J = 7.8 Hz, 2H), 3.23 (t, J = 8.5 Hz, 2H), 3.89 (s, 3H), 4.15 (t, J = 8.5 Hz, 2H), 6.97 (m, 2H),

7.49 (s, 1H), 7.54 (s, 1H), 7.78 (m, 2H). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 24.3, 27.4, 31.5, 45.7, 55.5, 113.5, 119.5, 125.8, 128.5, 128.9, 130.7, 132.2, 133.8, 144.9, 162.9, 167.9, 194.7.

8-[Hydroxy-(4-methoxy-phenyl)-methyl]-1,2,5,6-tetrahydro-pyrrolo[3,2,1-*ij*]quinolin-4-one (11a). The title compound was synthesized according to Method B using **11b** (570 mg, 1.85 mmol) and sodium hydride (140 mg, 3.70 mmol) in Methanol (10 mL) to yield a pale yellow solid (165 mg, 54%) after purification by flash chromatography on silica gel (EtOAc/PE, 1:10 to 2:1). R_f = 0.09 (EtOAc/PE, 1:1). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 2.32 (d, J = 3.4 Hz, 1H), 2.65 (t, J = 7.7 Hz, 2H), 2.93 (t, J = 7.7 Hz, 2H), 3.14 (t, J = 8.4 Hz, 2H), 3.80 (m, 4H), 4.06 (t, J = 8.4 Hz, 2H), 5.75 (s, br, 1H), 6.88 (m, 2H), 7.01 (s, 1H), 7.08 (s, 1H), 7.29 (m, 2H).

3-(4-Oxo-1,2,5,6-tetrahydro-4*H*-pyrrolo[3,2,1-*ij*]quinoline-8-carbonyl)-benzonitrile (12b). The title compound was synthesized according to Method D using **3c** (1.20 g, 2.60 mmol), 3-cyanobenzoyl chloride (0.86 g, 5.20 mmol) and dichlorobis(triphenyl phosphine)palladium(II) (0.18 g, 0.26 mmol) dry toluene (20 mL) to yield the crude product, which was purified by flash chromatography on silica gel (EtOAc/PE, 1:50 to 1:1) to yield a pale yellow solid (0.46 g, 58%). R_f = 0.12 (EtOAc/PE, 1:1). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 2.75 (t, J = 7.8 Hz, 2H), 3.04 (t, J = 7.8 Hz, 2H), 3.25 (t, J = 8.5 Hz, 2H), 4.16 (t, J = 8.5 Hz, 2H), 7.49 (s, 1H), 7.54 (s, 1H), 7.63 (t, J = 7.8 Hz, 1H), 7.85 (dt, J = 1.4, 7.8 Hz, 1H), 7.98 (m, 2H). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 24.2, 27.3, 31.3, 45.8, 112.7, 118.0, 119.9, 126.1, 128.9, 129.4, 129.4, 131.8, 133.0, 133.5, 134.9, 139.4, 146.1, 167.9, 193.3. MS (ESI) m/z = 279 $[M+H]^+$.

3-[Hydroxy-(4-oxo-1,2,5,6-tetrahydro-4*H*-pyrrolo[3,2,1-*ij*]quinolin-8-yl)-methyl]-benzonitrile (12a). The title compound was synthesized according to Method B using **12b** (455 mg, 1.50 mmol) and sodium hydride (114 mg, 3.00 mmol) in Methanol (10 mL) to yield a pale yellow solid (375 mg, 82%) after purification by flash chromatography on silica gel ($MeOH/CH_2Cl_2$, 0 to 1:50). R_f = 0.15 ($MeOH/CH_2Cl_2$, 1:20). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 2.55 (d, J = 3.3 Hz, 2H), 2.65 (t, J = 7.8 Hz, 2H), 2.94 (t, J = 7.8 Hz, 2H), 3.15 (t, J = 8.5 Hz, 2H), 4.06 (t, J = 8.5 Hz, 2H), 5.79 (d, J = 2.9 Hz, 1H), 6.97 (s, 1H), 7.04 (s, 1H), 7.44 (t, J = 7.8 Hz, 1H), 7.55 (dt, J = 1.3, 7.6 Hz, 1H), 7.63 (m, 1H), 7.69 (m, 1H). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 24.4, 27.7, 31.5, 45.4, 75.2, 112.5, 118.8, 120.4, 121.9, 124.2, 129.2, 129.6, 129.9, 130.6, 131.0, 138.5, 141.4, 145.4, 167.6.

4-(4-Oxo-1,2,5,6-tetrahydro-4*H*-pyrrolo[3,2,1-*ij*]quinoline-8-carbonyl)-benzonitrile (13b). The title compound was synthesized according to Method D using **3c** (1.50 g, 3.24 mmol), 4-cyanobenzoyl chloride (1.08 g, 6.49 mmol) and dichlorobis(triphenyl phosphine)palladium(II) (0.22 g, 0.32 mmol) dry toluene (20

mL) to yield the crude product, which was purified by flash chromatography on silica gel (EtOAc/PE, 1:10 to 1:1) to yield a yellow solid (0.71 g, 72%). R_f = 0.12 (EtOAc/PE, 1:1). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 2.74 (t, *J* = 7.8 Hz, 2H), 3.02 (t, *J* = 7.8 Hz, 2H), 3.24 (t, *J* = 8.5 Hz, 2H), 4.16 (t, *J* = 8.5 Hz, 2H), 7.50 (s, 1H), 7.55 (s, 1H), 7.80 (m, 4H). **4-[Hydroxy-(4-oxo-1,2,5,6-tetrahydro-4*H*-pyrrolo[3,2,1-*ij*]quinolin-8-yl)-methyl]-benzonitrile (13a).** The title compound was synthesized according to Method B using **13b** (704 mg, 2.33 mmol) and sodium hydride (177 mg, 4.66 mmol) in Methanol (15 mL) to yield a offwhite solid (475 mg, 67%) after purification by flash chromatography on silica gel (MeOH/CH_2Cl_2, 1:200 to 1:50). R_f = 0.03 (MeOH/CH_2Cl_2, 1:50). ^{1}H-NMR (500 MHz, DMSO-d_6): δ 2.50 (t, *J* = 7.7 Hz, 2H), 2.86 (t, *J* = 7.7 Hz, 2H), 3.08 (t, *J* = 8.5 Hz, 2H), 3.90 (t, *J* = 8.5 Hz, 2H), 5.71 (d, *J* = 3.9 Hz, 1H), 6.03 (d, *J* = 4.0 Hz, 1H), 7.02(s, 1H), 7.08 (s, 1H), 7.56 (m, 2H), 7.75 (m, 2H).

8-Benzoyl-1,2,5,6-tetrahydro-pyrrolo[3,2,1-*ij*]quinolin-4-one (14b). The title compound was synthesized according to Method A using **1c** (1.94 g, 10.8 mmol), $AlCl_3$ (3.99 g, 29.9 mmol) and benzoyl chloride (3.14 g, 22.3 mmol) in dry dichloromethane (50 mL) to yield the crude product, which was purified by flash chromatography on silica gel (hexanes/ EtOAc, 1:1, R_f = 0.14) to yield a yellow crystal (1.82 g, 61%). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 2.72 (t, *J* = 7.7 Hz, 2H), 3.01 (t, *J* = 7.7 Hz, 2H), 3.22 (t, *J* = 8.5 Hz, 2H), 4.14 (t, *J* = 8.5 Hz, 2H), 7.47 (m, 2H), 7.52 (s, 1H), 7.57 (m, 2H), 7.73 (m, 2H). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 24.2, 27.3, 31.4, 45.7, 119.5, 126.1, 128.2, 128.8, 128.9, 129.6, 131.9, 133.1, 138.3, 145.3, 167.8, 195.7.

8-(Hydroxy-phenyl-methyl)-1,2,5,6-tetrahydro-pyrrolo[3,2,1-ij]quinolin-4-one (14a). The title compound was synthesized according to Method B using **14b** (1.82 g, 6.56 mmol) and sodium hydride (378 mg, 10.0 mmol) in Methanol (50 mL) to yield a yellow solid (1.41 g, 77%). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 2.42 (s, br, 1H), 2.64 (t, *J* = 7.7 Hz, 2H), 2.95 (t, *J* = 7.7 Hz, 2H), 3.13 (t, *J* = 8.4 Hz, 2H), 4.04 (t, *J* = 8.4 Hz, 2H), 5.78 (s, 1H), 7.02 (s, 1H), 7.08 (s, 1H), 7.27 (m, 1H), 7.36 (m, 4H). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 24.4, 27.7, 31.5, 45.3, 76.2, 120.0, 121.8, 124.0, 126.3, 127.5, 128.5, 129.1, 139.6, 140.8, 144.0, 167.6.

8-(Furan-2-carbonyl)-1,2,5,6-tetrahydro-pyrrolo[3,2,1-*ij*]quinolin-4-one (15b). The title compound was synthesized according to Method D using **3c** (1.20 g, 2.60 mmol), 2-furoyl chloride (0.51 mL, 5.20 mmol) and dichlorobis(triphenyl phosphine)palladium(II) (0.18 g, 0.26 mmol) dry toluene (20 mL) to yield the crude product, which was purified by flash chromatography on silica gel (EtOAc/PE, 1:50 to 1:1) to yield an amber solid (0.38 g, 72%). R_f = 0.04 (EtOAc/PE, 1:1). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 2.73 (t, *J* = 7.8 Hz, 2H), 3.04 (t, *J* = 7.8 Hz, 2H), 3.25 (t, *J* = 8.5 Hz, 2H), 4.14 (t, *J* = 8.5 Hz, 2H), 6.59 (dd, *J* = 1.7, 3.5 Hz, 1H),

7.23 (d, *J* = 3.4 Hz, 1H), 7.69 (m, 1H), 7.74 (s, 1H), 7.78 (s, 1H). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 24.3, 27.4, 31.4, 45.7, 112.1, 119.7, 119.7, 125.4, 128.0, 129.1, 132.7, 145.5, 146.6, 152.7, 167.8, 181.3.

8-(Furan-2-yl-hydroxy-methyl)-1,2,5,6-tetrahydro-pyrrolo[3,2,1-*ij*]quinolin-4-one (15a). The title compound was synthesized according to Method B using **15b** (360 mg, 1.35 mmol) and sodium hydride (102 mg, 2.69 mmol) in Methanol (10 mL) to yield an amber solid (344 mg, 95%) after purification by flash chromatography on silica gel ($MeOH/CH_2Cl_2$, 1:200 to 1:50). R_f = 0.30 ($MeOH/CH_2Cl_2$, 1:20). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 2.55 (d, *J* = 4.2 Hz, 1H), 2.66 (t, *J* = 7.8 Hz, 2H), 2.95 (t, *J* = 7.8 Hz, 2H), 3.17 (t, *J* = 8.4 Hz, 2H), 4.07 (t, *J* = 8.4 Hz, 2H), 5.76 (d, *J* = 4.0 Hz, 1H), 6.18 (m, 1H), 6.33 (dd, *J* = 1.8, 3.2 Hz, 1H), 7.08 (s, 1H), 7.15 (s, 1H), 7.38 (m, 1H). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 24.4, 27.7, 31.5, 45.4, 70.2, 107.0, 110.2, 120.0, 121.9, 124.1, 129.1, 136.4, 141.3, 142.5, 156.1, 167.6.

8-(Thiophene-2-carbonyl)-1,2,5,6-tetrahydro-pyrrolo[3,2,1-*ij*]quinolin-4-one (16b). The title compound was synthesized according to Method D using **3c** (1.20 g, 2.60 mmol), 2-thiophene carbonyl chloride (0.56 mL, 5.20 mmol) and dichlorobis(triphenyl phosphine)palladium(II) (0.18 g, 0.26 mmol) dry toluene (20 mL) to yield the crude product, which was purified by flash chromatography on silica gel (EtOAc/PE, 1:50 to 4:5) to yield a yellow solid (0.50 g, 67%). R_f = 0.05 (EtOAc/PE, 1:1). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 2.74 (t, *J* = 7.8 Hz, 2H), 3.04 (t, *J* = 7.8 Hz, 2H), 3.25 (t, *J* = 8.5 Hz, 2H), 4.15 (t, *J* = 8.5 Hz, 2H), 7.16 (dd, *J* = 3.8, 4.9 Hz, 1H), 7.60 (s, 1H), 7.63 (dd, *J* = 1.0, 3.8 Hz, 1H), 7.65 (s, 1H) , 7.69 (dd, *J* = 1.1, 4.9 Hz, 1H). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 24.2, 27.4, 31.4, 45.7, 119.7, 125.2, 127.8, 127.9, 129.1, 133.6, 133.7, 134.0, 143.8, 145.2, 167.8, 167.8, 187.0.

8-(Hydroxy-thiophen-2-yl-methyl)-1,2,5,6-tetrahydro-pyrrolo[3,2,1-*ij*]quinolin-4-one (16a). The title compound was synthesized according to Method B using **16b** (480 mg, 1.69 mmol) and sodium hydride (129 mg, 3.39 mmol) in Methanol (15 mL) to yield an amber solid (400 mg, 83%) after purification by flash chromatography on silica gel ($MeOH/CH_2Cl_2$, 0 to 1:50). R_f = 0.22 ($MeOH/CH_2Cl_2$, 1:20). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 2.66 (t, *J* = 7.8 Hz, 2H), 2.95 (t, *J* = 7.8 Hz, 2H), 3.16 (t, *J* = 8.4 Hz, 2H), 4.07 (t, *J* = 8.4 Hz, 2H), 6.00 (d, *J* = 3.1 Hz, 1H), 6.91 (d, *J* = 3.5 Hz, 1H), 7.00 (dd, *J* = 3.5, 5.0 Hz, 1H), 7.09 (s, 1H), 7.16 (s, 1H), 7.26 (m, 2H). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 24.4, 27.7, 31.5, 45.4, 70.4, 120.0, 121.5, 123.8, 124.5, 125.3, 126.7, 129.2, 138.9, 141.1, 148.5, 167.7.

8-[(1-1*H*-imidazol-1-yl-ethyl)]-1,2,5,6-tetrahydro-pyrrolo[3,2,1-*ij*]quinolin-4-one (1). The title compound was synthesized according to Method C using **1a** (1.80 g, 8.30 mmol), imidazole (4.52 g, 66.4 mmol), $SOCl_2$

(1.20 mL, 16.6 mmol), and dry THF (40 mL). The crude product was purified by two subsequent crystallizations from acetone to yield pale yellow crystals (488 mg, 22%). mp 196–198 °C, . ^{1}H-NMR (500 MHz, $CDCl_3$): δ 1.82 (d, *J* = 6.9 Hz, 3H), 2.65 (t, *J* = 7.7 Hz, 2H), 2.92 (t, *J* = 7.7 Hz, 2H), 3.14 (t, *J* = 8.5 Hz, 2H), 4.07 (t, *J* = 8.5 Hz, 2H), 5.27 (q, *J* = 6.9 Hz, 1H), 6.79 (s, 1H), 6.85 (s, 1H), 6.91 (s, 1H), 7.07 (s, 1H), 7.58 (s, 1H). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 22.3, 24.4, 27.7, 31.5, 45.4, 56.6, 117.9, 120.4, 121.2, 123.5, 129.3, 129.6, 135.9, 136.9, 141.4, 167.5. MS (ESI) *m/z* = 268 $[M+H]^+$.

8-[1-(1*H*-imidazol-1-yl)propyl]-1,2,5,6-tetrahydropyrrolo[3,2,1-ij]quinolin-4-one (2). The title compound was synthesized according to Method C using **2a** (0.63 mg, 2.73mmol), imidazole (2.94 g, 43.2 mmol), $SOCl_2$ (0.78 mL, 10.7 mmol) and dry THF (25 mL). The crude product was purified by flash chromatography (EtOAc/MeOH, 9:1, R_f = 0.11) to yield white solids (0.20 g, 26%). mp 194–196 °C. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 0.93 (t, *J* = 7.2, 3H), 2.13–2.25 (m, 2H), 2.65 (t, *J* = 7.9, 2H), 2.93 (t, *J* = 7.9, 2H), 3.14 (t, *J* = 8.5, 2H), 4.06 (t, *J* = 8.5, 2H), 4.90–4.94 (t, ^{3}J = 7.6, 1H), 6.82 (s, 1H), 6.90 (s, 1H), 6.92 (s, 1H), 7.06 (s, 1H), 7.59 (s, 1H). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 11.4, 24.6, 27.9, 29.0, 31.7, 45.6, 63.6, 117.8, 120.6, 121.9, 124.3, 129.6, 129.8, 135.8, 136.4, 141.7, 167.7. MS (ESI) *m/z* = 282 $[M+H]^+$.

8-[Cyclopropyl(1*H*-imidazol-1-yl)methyl]-1,2,5,6-tetrahydropyrrolo[3,2,1-*ij*]quinolin-4-one (4). The title compound was synthesized according to Method C using **4a** (0.56 g, 2.30 mmol), imidazole (1.88 g, 27.6 mmol), $SOCl_2$ (0.50 mL, 6.90 mmol) and dry THF (20 mL). The crude product was purified by flash chromatography ($MeOH/CH_2Cl_2$, 0 to 1:50) to yield white crystals (0.50 g, 74%). mp 146–147 °C, R_f = 0.14. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 0.42–0.50 (m, 2H), 0.76–0.85 (m, 2H), 1.46–1.54 (m, 1H), 2.66 (t, *J* = 7.8 Hz, 2H), 2.93 (t, *J* = 7.8 Hz, 2H), 3.15 (t, *J* = 8.5 Hz, 2H), 4.08 (t, *J* = 8.5 Hz, 2H), 4.30 (d, *J* = 9.3 Hz, 1H), 6.82 (s, 1H), 6.90 (s, 1H), 6.95 (s, 1H), 7.07 (s, 1H), 7.67 (s, 1H). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 4.8, 5.3, 16.6, 24.4, 27.7, 31.5, 45.4, 66.3, 118.3, 120.3, 121.8, 124.1, 129.3, 129.4, 135.6, 136.4, 141.4, 167.5. MS (ESI) *m/z* = 226 $[M\text{-imidazole}]^+$. The racemate **4** was separated by preparative HPLC on chiral stationary phase (NucleoCel Delta column, 0.8 cm × 25 cm, 45% hexane/ethanol, 1.0 mL/min) to yield **4-1** (99.6% ee and t_R = 19.2 min by analytical HPLC using 30% hexane/ethanol) and **4-2** (93.3% ee and t_R = 22.9 min by analytical HPLC using 30% hexane/ethanol).

8-[Cyclobutyl(1*H*-imidazol-1-yl)methyl]-1,2,5,6-tetrahydropyrrolo[3,2,1-*ij*]quinolin-4-one (5). The title compound was synthesized according to Method C using **5a** (0.20 g, 0.78 mmol), imidazole (0.85 g, 12.4

mmol), $SOCl_2$ (0.23 mL, 3.11 mmol) and dry THF (10 mL). The crude product was purified by flash chromatography ($MeOH/CH_2Cl_2$, 0 to 1:50) to yield white crystals (0.20 g, 84%). mp 120–122 °C, R_f = 0.14. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 1.76–1.99 (m, 4H), 2.01–2.07 (m, 1H), 2.14–2.21 (m, 1H), 2.65 (t, *J* = 7.8 Hz, 2H), 2.92 (t, *J* = 7.8 Hz, 2H), 3.08 (m, 1H), 3.13 (t, *J* = 8.5 Hz, 2H), 4.06 (t, *J* = 8.5 Hz, 2H), 4.93 (d, *J* = 10.4 Hz, 1H), 6.80 (s, 1H), 6.85 (s, 1H), 6.87 (s, 1H), 7.03 (s, 1H), 7.51 (s, 1H). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 17.5, 24.5, 26.3, 26.9, 27.8, 31.5, 39.8, 45.4, 67.3, 117.8, 120.3, 122.3, 124.4, 129.4, 129.5, 134.4, 136.2, 141.7, 167.3. MS (ESI) *m/z* = 240 [M-imidazole]$^+$.

8-[(2-Fluorophenyl)(1*H*-imidazol-1-yl)methyl]-1,2,5,6-tetrahydropyrrolo[3,2,1-*ij*]quinolin-4-one (7). The title compound was synthesized according to Method C using **7a** (0.16 g, 0.55 mmol), imidazole (0.6 1g, 8.88 mmol), $SOCl_2$ (0.16 mL, 2.22 mmol) and dry THF (10 mL). The crude product was purified by flash chromatography ($MeOH/CH_2Cl_2$, 0 to 1:10) to yield pale yellow solids (0.10 g, 55%). mp 175–177 °C, R_f = 0.11. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 2.67 (t, *J* = 7.8 Hz, 2H), 2.91 (t, *J* = 7.8 Hz, 2H), 3.14 (t, *J* = 8.5 Hz, 2H), 4.08 (t, *J* = 8.5 Hz, 2H), 6.73 (s, 1H), 6.74 (s, 1H), 6.80 (s, 1H), 6.85 (s, 1H), 6.88 (m, 1H), 7.08–7.16 (m, 3H), 7.35 (m, 1H), 7.41 (s, 1H). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 24.3, 27.6, 31.4, 45.4, 58.5 (d, $^3J_{C,F}$ = 4.0 Hz), 115.8 (d, $^2J_{C,F}$ = 21.2 Hz), 119.3, 120.0, 123.0, 124.6 (d, $^4J_{C,F}$ = 3.5 Hz), 125.4, 127.0 (d, $^3J_{C,F}$ = 13.1 Hz), 128.7 (d, $^4J_{C,F}$ = 2.9 Hz), 129.5, 129.6, 130.3 (d, $^3J_{C,F}$ = 8.3 Hz), 133.0, 137.3, 141.8, 160.0 (d, $^1J_{C,F}$ = 249 Hz), 167.5. MS (ESI) *m/z* = 280 [M-imidazole]$^+$. The racemate **7** was separated by preparative HPLC on chiral stationary phase (NucleoCel Delta column, 0.8 cm × 25 cm, 20% hexane/isopropanol, 1.0 mL/min) to yield **7-1** (100% ee and t_R = 22.2 min by analytical HPLC using 20% hexane/isopropanol) and **7-2** (71.4% ee and t_R = 27.3 min by analytical HPLC using 20% hexane/isopropanol).

8-[(3-Fluorophenyl)(1*H*-imidazol-1-yl)methyl]-1,2,5,6-tetrahydropyrrolo[3,2,1-*ij*]quinolin-4-one (8). The title compound was synthesized according to Method C using **8a** (230 mg, 0.77 mmol), imidazole (843 mg, 8.88 mmol), $SOCl_2$ (0.22 mL, 3.09 mmol) and dry THF (10 mL). The crude product was purified by flash chromatography ($MeOH/CH_2Cl_2$, 1:200 to 1:50) to yield pale yellow solids (60 mg, 22%). mp 194–196 °C, R_f = 0.12. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 2.68 (t, *J* = 7.8 Hz, 2H), 2.92 (t, *J* = 7.8 Hz, 2H), 3.15 (t, *J* = 8.5 Hz, 2H), 4.09 (t, *J* = 8.5 Hz, 2H), 6.43 (s, 1H), 6.75 (s, 1H), 6.78 (m, 1H), 6.81 (s, 1H), 6.84 (s, 1H), 6.88 (m, 1H), 7.04 (m, 1H), 7.11 (s, 1H), 7.34 (m, 1H), 7.41 (s, 1H). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 24.4, 27.7, 31.4, 45.4, 64.4, 114.9 (d, $^2J_{C,F}$ = 22.8 Hz), 115.4 (d, $^2J_{C,F}$ = 21.1 Hz), 120.5, 123.3, 123.4, 123.4, 125.7,

129.7, 129.7, 130.5 (d, $^3J_{C,F}$ = 8.2 Hz), 133.6, 141.9, 142.0, 142.0, 163.0 (d, $^1J_{C,F}$ = 248 Hz), 167.5. MS (ESI) *m/z* = 281 [M-imidazole]$^+$.

8-[(4-Fluorophenyl)(1*H*-imidazol-1-yl)methyl]-1,2,5,6-tetrahydropyrrolo[3,2,1-*ij*]quinolin-4-one (9). The title compound was synthesized according to Method C using **9a** (0.35 g, 1.18 mmol), imidazole (1.28 g, 18.8 mmol), $SOCl_2$ (0.34 mL, 4.72 mmol) and dry THF (12 mL). The crude product was purified by flash chromatography ($MeOH/CH_2Cl_2$, 0 to 1:50) to yield pale yellow solids (0.30 g, 73%). mp 201–203 °C, R_f = 0.11. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 2.67 (t, *J* = 7.8 Hz, 2H), 2.92 (t, *J* = 7.8 Hz, 2H), 3.15 (t, *J* = 8.5 Hz, 2H), 4.09 (t, *J* = 8.5 Hz, 2H), 6.46 (s, 1H), 6.73 (s, 1H), 6.78 (s, 1H), 6.85 (s, 1H), 7.06 (s, 2H), 7.07 (s, 2H), 7.13 (s, 1H), 7.47 (s, 1H). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 24.4, 27.7, 31.4, 45.4, 64.5, 116.0 (d,$^2J_{C,F}$ = 21.8 Hz), 119.3, 120.5, 123.1, 125.5, 128.9, 129.6 (d, $^3J_{C,F}$ = 8.2 Hz), 129.8, 133.9, 135.0 (d, $^4J_{C,F}$ = 3.5 Hz), 137.0, 141.9, 162.5 (d, $^1J_{C,F}$ = 248 Hz), 167.5. MS (ESI) *m/z* = 281 [M-imidazole]$^+$.

8-[(1*H*-imidazol-1-yl)(3-methoxyphenyl)methyl]-1,2,5,6-tetrahydropyrrolo[3,2,1-*ij*]quinolin-4-one (10). The title compound was synthesized according to Method C using **10a** (0.40 g, 1.29 mmol), imidazole (1.06 g, 15.5 mmol), $SOCl_2$ (0.28 mL, 3.88 mmol) and dry THF (20 mL). The crude product was purified by flash chromatography ($MeOH/CH_2Cl_2$, 0 to 1:50) to yield white crystals (0.42 g, 87%). mp 65–67 °C, R_f = 0.10. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 2.66 (t, *J* = 7.8 Hz, 2H), 2.91 (t, *J* = 7.8 Hz, 2H), 3.13 (t, *J* = 8.5 Hz, 2H), 3.76 (s, 3H), 4.08 (t, *J* = 8.5 Hz, 2H), 6.40 (s, 1H), 6.61 (t, *J* = 2.0 Hz, 1H), 6.67 (d, *J* = 7.6 Hz,1H), 6.75 (s, 1H), 6.81 (s, 1H), 6.84 (m, 1H), 6.87 (dd, *J* = 2.5, 8.2 Hz, 1H), 7.08 (m, 1H), 7.27 (t, *J* = 7.8 Hz, 1H), 7.40 (s, 1H). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 24.3, 27.6, 31.4, 45.4, 55.3, 64.8, 113.2, 114.0, 119.3, 120.1, 120.3, 123.2, 125.6, 129.4, 129.5, 129.9, 134.2, 137.3, 140.9, 141.6, 160.0, 167.5. MS (ESI) *m/z* = 360.60 [M+H]$^+$.

8-[(1*H*-imidazol-1-yl)(4-methoxyphenyl)methyl]-1,2,5,6-tetrahydropyrrolo[3,2,1-*ij*]quinolin-4-one (11). The title compound was synthesized according to Method C using **11a** (340 mg, 1.10 mmol), imidazole (1.20 g, 17.6 mmol), $SOCl_2$ (0.32 mL, 4.40 mmol) and dry THF (15 mL). The crude product was purified by flash chromatography ($MeOH/CH_2Cl_2$, 0 to 1:50) to yield white crystals (30 mg, 9%). mp 54–56 °C, R_f = 0.06. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 2.66 (t, *J* = 7.8 Hz, 2H), 2.91 (t, *J* = 7.8 Hz, 2H), 3.14 (t, *J* = 8.5 Hz, 2H), 3.81 (s, 3H), 4.08 (t,^3J = 8.5 Hz, 2H), 6.40 (s, 1H), 6.72 (s, 1H), 6.78 (s, 1H), 6.86 (m, 1H), 6.88 (m, 2H), 7.02 (m, 2H), 7.11 (s, 1H), 7.43 (s, 1H). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 24.4, 27.7, 31.4, 45.4, 55.3, 64.6, 114.3,

114.5, 116.6, 117.1, 120.4, 123.0, 125.3, 129.2, 129.6, 131.1, 134.7, 141.6, 159.6, 167.5. MS (ESI) *m/z* = 293 [M-imidazole]$^+$.

3-[(2,4,5,6-Tetrahydro-4-oxo-1H-pyrrolo[3,2,1-*ij*]quinolin-8-yl)(1*H*-imidazol-1-yl)methyl]Benzonitrile (12). The title compound was synthesized according to Method C using **12a** (180 mg, 0.59 mmol), imidazole (644 mg, 9.46 mmol) and $SOCl_2$ (0.17 mL, 2.37 mmol) and dry THF (10 mL). The crude product was purified by flash chromatography ($MeOH/CH_2Cl_2$, 0 to 1:20) to yield white solids (30 mg, 14%). mp 201–202 °C, R_f = 0.07. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 2.68 (t, *J* = 7.8 Hz, 2H), 2.93 (t, *J* = 7.8 Hz, 2H), 3.16 (t, *J* = 8.5 Hz, 2H), 4.10 (t, *J* = 8.5 Hz, 2H), 6.48 (s, 1H), 6.73 (s, 1H), 6.78 (s, 1H), 6.83 (s, 1H), 7.14 (s, 1H), 7.30 (d, *J* = 7.9 Hz, 1H), 7.34 (s, 1H), 7.42 (s, 1H), 7.50 (t, *J* = 8.0 Hz, 1H) , 7.64 (d, *J* = 7.7 Hz, 1H). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 24.3, 27.6, 31.3, 45.4, 64.2, 113.2, 118.1, 120.7, 123.4, 125.7, 129.8, 130.0, 131.1, 131.9, 132.0, 132.7, 141.2, 142.2, 167.5. MS (ESI) *m/z* = 287 [M-imidazole]$^+$.

4-[(2,4,5,6-Tetrahydro-4-oxo-1H-pyrrolo[3,2,1-*ij*]quinolin-8-yl)(1*H*-imidazol-1-yl)methyl]Benzonitrile (13). The title compound was synthesized according to Method C using **13a** (460 mg, 1.51 mmol), imidazole (1.22 g, 18.0 mmol), $SOCl_2$ (0.33 mL, 4.53 mmol) and dry THF (20 mL). The crude product was purified by flash chromatography ($MeOH/CH_2Cl_2$, 1:500 to 1:50) to yield white solids (302 mg, 56%). mp 114–116 °C, R_f = 0.12. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 2.67 (t, *J* = 7.8 Hz, 2H), 2.92 (t, *J* = 7.8 Hz, 2H), 3.15 (t, *J* = 8.5 Hz, 2H), 4.09 (t, *J* = 8.5 Hz, 2H), 6.49 (s, 1H), 6.74 (s, 1H), 6.79 (s, 1H), 6.82 (s, 1H), 7.13 (s, 1H), 7.16 (d, *J* = 8.3 Hz, 2H),7.40 (s, 1H), 7.66 (d, *J* = 8.3 Hz, 2H). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 24.3, 27.6, 31.3, 45.4, 64.5, 112.4, 118.1, 119.0, 120.7, 123.5, 126.0, 128.2, 130.0, 130.0, 132.6, 132.7, 137.1, 142.2, 144.8, 167.4. MS (ESI) *m/z* = 356 [M+H]$^+$.

8-[(1*H*-imidazol-1-yl)(phenyl)methyl]-1,2,5,6-tetrahydropyrrolo[3,2,1-*ij*]quinolin-4-one (14). The title compound was synthesized according to Method C using **14a** (1.41 g, 5.06 mmol), imidazole (2.72 g, 40.0 mmol), $SOCl_2$ (0.73 mL, 10.0 mmol) and dry THF (40 mL). The crude product was purified by two subsequent crystallizations from acetone to yield colorless needles (385 mg, 23%). mp185–187 °C. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 2.67 (t, *J* = 7.7 Hz, 2H), 2.92 (t, *J* = 7.7 Hz, 2H), 3.14 (t, *J* = 8.5 Hz, 2H), 4.09 (t, *J* = 8.5 Hz, 2H), 6.45 (s, 1H), 6.75 (s, 1H), 6.81 (s, 1H), 6.85 (s, 1H), 7.09 (m, 3H), 7.33–7.39 (m, 3H), 7.41 (s, 1H). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 24.4, 27.7, 31.4, 45.4, 65.0, 119.3, 120.4, 123.3, 125.6, 127.8, 128.4, 128.9, 129.4, 129.6, 134.4, 137.3, 139.3, 141.6, 167.5. MS (ESI) *m/z* = 330 [M+H]$^+$.

8-(Furan-2-yl(1*H*-imidazol-1-yl)methyl)-1,2,5,6-tetrahydropyrrolo[3,2,1-*ij*]quinolin-4-one (15). The title compound was synthesized according to Method C using **15a** (200 mg, 0.74 mmol), imidazole (809 mg, 11.9 mmol), $SOCl_2$ (0.22 mL, 2.97 mmol) and dry THF (10 mL). The crude product was purified by flash chromatography ($MeOH/CH_2Cl_2$, 0 to 3:100) to yield yellow solids (65 mg, 28%). mp 151–153 °C, R_f = 0.08. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 2.67 (t, *J* = 7.8 Hz, 2H), 2.92 (t, *J* = 7.8 Hz, 2H), 3.15 (t, *J* = 8.5 Hz, 2H), 4.08 (t, *J* = 8.5 Hz, 2H), 6.17 (d, *J* = 3.3 Hz, 1H), 6.38 (m, 2H), 6.78 (s, 1H), 6.84 (s, 1H), 6.89 (s, 1H), 7.08 (s, 1H), 7.45 (s, 2H). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 24.3, 27.6, 31.4, 45.4, 58.8, 110.2, 110.5, 118.7, 120.4, 122.3, 124.7, 129.4, 129.6, 132.6, 136.8, 141.8, 143.4, 151.4, 167.5. MS (ESI) *m/z* = 252 [M-imidazole]$^+$.

8-[(1*H*-imidazol-1-yl)(thiophen-2-yl)methyl]-1,2,5,6-tetrahydropyrrolo[3,2,1-*ij*]quinolin-4-one (16). The title compound was synthesized according to Method C using **16a** (200 mg, 0.70 mmol), imidazole (762 mg, 11.2 mmol), $SOCl_2$ (0.20 mL, 2.80 mmol) and dry THF (10 mL). The crude product was purified by flash chromatography ($MeOH/CH_2Cl_2$, 0 to 3:100) to yield yellow solids (150 mg, 64%). mp 169–171 °C, R_f = 0.15. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 2.67 (t, *J* = 7.8 Hz, 2H), 2.92 (t, *J* = 7.8 Hz, 2H), 3.15 (t, *J* = 8.5 Hz, 2H), 4.08 (t, *J* = 8.5 Hz, 2H), 6.61 (s, 1H), 6.82 (s, 1H), 6.86 (dt, *J* = 1.0, 3.5Hz, 1H), 6.88 (s, 1H), 6.91 (m, 1H), 7.00 (dd, *J* = 3.6, 5.1 Hz, 1H), 7.09 (s, 1H), 7.34 (dd, *J* = 1.2, 5.1 Hz, 1H), 7.48 (s, 1H). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 24.3, 27.7, 31.4, 45.4, 60.5, 118.9, 120.4, 122.3, 124.7, 126.7, 127.0, 127.5, 129.5, 129.6, 134.8, 136.9, 141.8, 142.5, 167.5. MS (ESI) *m/z* = 268 $[M\text{-imidazole}]^+$.

8-[1-(1*H*-imidazol-1-yl)vinyl]-1,2,5,6-tetrahydropyrrolo[3,2,1-*ij*]quinolin-4-one (6); 8-[1,1-Di(1*H*-imidazol-1-yl)ethyl]-1,2,5,6-tetrahydropyrrolo[3,2,1-*ij*]quinolin-4-one (17). To a solution of imidazole (2.28 g, 33.4 mmol) in dry dichloromethane (15 mL) was added $SOCl_2$ (0.40 mL, 5.6 mmol) under an atmosphere of nitrogen at 0 °C. The mixture was stirred for 30 min, and **1b** (1.00 g, 4.60 mmol) was added. The mixture was stirred at ambient temperature for 96 h, consequently neutralized with aqueous $NaHCO_3$ and extracted with $CHCl_3$ (3 x 15 mL). The extracts were washed with water and brine, dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by flash chromatograph ($MeOH/CH_2Cl_2$, 0 to 1:50) to yield **6** as pale yellow solids (28 mg, 2%). mp 125–126 °C, R_f = 0.11. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 2.69 (t, *J* = 7.7 Hz, 2H), 2.96 (t, *J* = 7.7 Hz, 2H), 3.18 (t, *J* = 8.5 Hz, 2H), 4.11 (t, *J* = 8.5 Hz, 2H), 5.21 (d, *J* = 9.9 Hz, 2H), 6.97 (s, 1H), 7.03 (m, 2H), 7.13 (s, 1H), 7.62 (s, 1H). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 24.3, 27.5, 31.4, 45.5, 105.2, 119.3, 120.2, 122.5, 124.9, 129.4, 129.7, 131.2, 137.2, 142.8, 143.2, 167.5. MS (ESI) *m/z* = 198 $[M\text{-imidazole}]^+$. Subsequent elution ($MeOH/CH_2Cl_2$, 1:50 to 1:10) yielded **17** as pale yellow solids (330 mg, 22%). mp 189 °C, R_f = 0.04. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 2.53 (s, 3H), 2.67 (t, *J* = 7.8 Hz, 2H), 2.91 (t, *J* = 7.8 Hz, 2H), 3.14 (t, *J* = 8.5 Hz, 2H), 4.10 (t, *J* = 8.5 Hz, 2H), 6.52 (s, 1H), 6.56 (s, 1H), 6.82 (s, 2H), 7.13 (s, 2H), 7.35 (s, 2H). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 24.4, 27.6, 30.3, 31.3, 45.5, 75.9, 118.0, 120.4, 121.2, 123.5, 129.8, 130.3, 135.6, 136.2, 142.8, 167.4. MS (ESI) *m/z* = 266 $[M\text{-imidazole}]^+$.

2. HPLC purity control of all final compounds.

The Surveyor®-LC-system consisted of a pump, an autosampler, and a PDA detector. Mass spectrometry was performed on a TSQ® Quantum (Thermo Electron Corporation, Dreieich, Germany). The triple quadrupole mass spectrometer was equipped with an electrospray interface (ESI). The system was operated by the standard software Xcalibur®.

A RP C18 NUCLEODUR® 100-5 (125 × 3 mm) column (Macherey-Nagel GmbH, Duehren, Germany) was used as stationary phase. All solvents were HPLC grade.

In a gradient run the percentage of acetonitrile (containing 0.1% triflouro-acetic acid) in water was increased from an initial concentration of 3% at 0 min to 100% at 15 min and kept at 100% for 3 min.

The injection volume was 10 µl and flow rate was set to 350 µl/min. MS analysis was carried out at a spray voltage of 3800 V, a capillary temperature of 350 °C and a source CID of 10 V. Spectra were acquired in positive mode from 100 to 1000 m/z and full scan UV-mode. In some cases APC ionization had to be applied.

Comp.	RT (min)	Purity [%]
1	6.74	99.0%
2	6.01	95.1%
3	5.57	98.2%
4	5.24	95.6%
5	7.82	99.9%
6	7.86	98.0%
7	6.07	99.7%
8	6.25	98.2%
9	6.36	99.7%
10	6.51	97.3%
11	6.26	95.3%
12	7.19	99.9%
13	5.84	99.0%
14	9.01	98.8%
15	5.51	96.8%
16	5.92	98.0%
17	8.79	99.3%

4 Summary and Conclusions

4.1 CYP11B2 Inhibitors

Our previously designed CYP11B2 inhibitors with a naphthalene core showed strong potentcy and good selectivity toward CYP11B1[63-66]. However, high inhibition of an important hepatic enzyme, CYP1A2, was observed[65,66]. In order to improve the selectivity toward CYP1A2, structural modifications regarding aromaticity, planarity, and structural flexibility/rigidity were carried out on the core structure by altering cycle size, presence of H-bond forming groups and removal of bridge bonds. In this study, 21 analogues of 3-pyridinyl substituted aliphatic cycles were designed, synthesized and biologically evaluated as novel CYP11B2 inhibitors. Among them, compounds **I-2**, **I-4**, **I-12**, **I-13** and **I-14** (Figure 16) were identified as potent CYP11B2 inhibitors with good selectivity toward CYP11B1. On one hand, the presence of H-bond forming groups was observed to reduce inhibitory potency. On the other hand, an α-double bond together with a H-bond forming group in medium size cycles promoted inhibitory activity and selectivity, whereas deterioration was observed for medium size cycles without H-bond forming groups. This phenomenon is probabably due to the fact that the binding mode altered when H-bond forming groups interacted with some amino acid residues. Furthermore, flexible 8 to 10 membered medium or bicyclic rings are appropriate for the binding site, whereas flexible 12-membered ring or rigid bicyclic analogues are not tolerated. In addition, the design concept employed in this study through dearomatisation of the lipophilic core and destruction of planarity to reduce CYP1A2 inhibition was proven successful. For the five most potent and selective CYP11B2 inhibitors, the CYP1A2 inhibition was significantly decreased compared to their aromatic precursors. Since selectivity toward hepatic CYP enzymes is always a key issue for safety reasons associated with drug discovery, the results demonstrated in this study can be helpful in the optimization of steroidogenic CYP inhibitors aimed at improvement of selectivity toward CYP1A2.

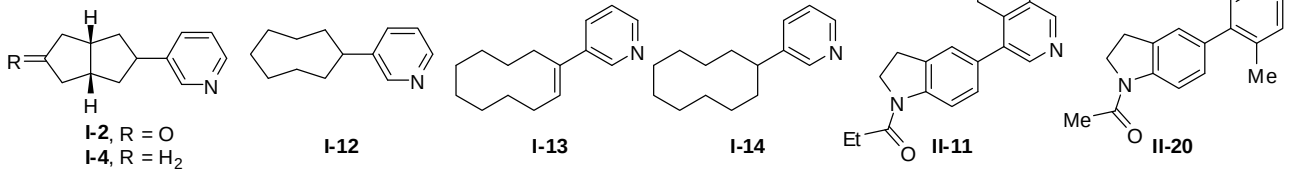

Figure 16. CYP11B2 inhibitors.

Moreover, on the basis of a dihydroquinolinone core[67, 69] and a dihydropyrroloquinolinone core[70], a series of N containing heterocycle substituted indolines and indoles were designed, synthesized and identified as highly potent and selective CYP11B2 inhibitors via ligand-based approach. Especially, compounds **II-11** and **II-20** (Figure 16) showed IC_{50} values around 2 nM and SFs around 170. It is notable that they showed no inhibition of other steroidogenic enzymes such as CYP17 and CYP19. These compounds can be considered as candidates for further evaluation in vivo. In this study, it has been found that bulky substituents at the amido moiety dramatically reduced inhibitory potency. The methyl group turned out to be the most suitable

one. Introduction of electron donating groups, such as methyl, methoxy and phenyl on the pyridyl significantly increased CYP11B2 inhibition. Finally, dehydrogenation of the indoline core to indole led to an increase in CYP11B2 inhibition, but no improvement of selectivity.

4.2 Dual Inhibitors of CYP19 / CYP11B2

The same inhibition mechanism of heme iron complexing CYP enzyme inhibitors and a certain homology among this superfamily become advantegous for the design of dual inhibitors. Through combination of the key structure features of the target enzyme inhibitors, a series of potent dual CYP19 / CYP11B2 inhibitors were designed, synthesized and tested. 3-Pyridinyl, a favorable N containing heterocycle for CYP11B2 inhibitors, has been found not to be suitable for this purpose, whereas 4-pyridinyl led to potent dual inhibitors. The substituents on the CH bridge showed significant influence on the inhibitory activity, in which contradictory SARs were commonly observed for the inhibition of these two enzymes. For example, when alkyl groups were introduced, the enhancement of bulkiness increased CYP19 inhibition but reduced that of CYP11B2. Besides, substituted phenyl groups were introduced instead of alkyl groups, resulting in compounds with strong inhibition toward both enzymes. Similarly, contradictory SARs were observed when methoxy or fluoro at the phenyl moiety were shifted from the 2- or 3-position into the 4-position, leading to decrease in CYP19 inhibition but an increase in CYP11B2 inhibition. Contrarily, chloro and methyl substitution led to potent dual inhibitors of CYP19 and CYP11B2 regardless their positions on the phenyl ring. It has been observed that 3-substituted Ph compounds were more potent toward CYP19, whereas 4-substituted Ph derivatives showed a stronger inhibition of CYP11B2, and compounds with 3-substituted Ph were more selective than the corresponding 4-substituted Ph analogues. Moreover, the resolution of a racemic mixture led to an increase in CYP19 inhibition but to a reduction of CYP11B2 inhibition and selectivity regarding CYP11B1 for one enantiomer; while for the other one opposite results were observed. The compromise of these conflicts led to compounds **III-1[9**, **III-20**, **III-23** and **III-25** as potent and selective dual inhibitors of CYP19 and CYP11B2. Especially compound **III-25**, exhibiting IC_{50} values of 32 and 41 nM for CYP19 and CYP11B2, respectively, and a SF of 33 over CYP11B1, is deemed to be superior to the leads fadrozole which is an unselective CYP19 inhibitor showing potent inhibition of CYP11B2 and CYP11B1. This compound could be a drug candidate for treatment of BC patients with risk of CVD after further evaluation in vivo.

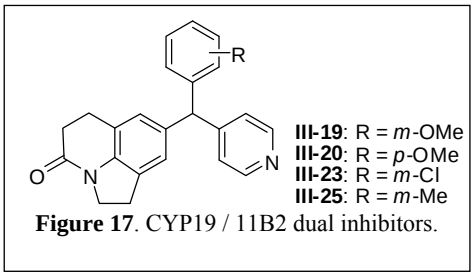

Figure 17. CYP19 / 11B2 dual inhibitors.

4.3 CYP11B1 Inhibitors

Starting from etomidate and 8-(isoquinolin-4-yl)-5,6-dihydro-1*H*-pyrrolo[3,2,1-*ij*]quinolin-4(2*H*)-one (Figure 18), modification of the etomidate scaffold by replacement of the phenyl group by the tetrahydropyrroloquinolinone group led to a series of imidazol-1-ylmethyl substituted 1,2,5,6-tetrahydro-pyrrolo[3,2,1-ij]quinolin-4-ones. Through optimization of substituents on the CH bridge between the tetrahydropyrroloquinolinone core and the imidazolyl, the selectivity toward CYP11B2 was improved. The

inhibitory potency increased from about 20 nM to less than 5 nM with increasing bulkiness of the aliphatic chains. However, steric limitations were observed when substituted aromatic phenyl groups were introduced. Moreover, compounds with substitutents at the *para*- position of the phenyl group showed improved inhibition and selectivity compared to non- or *meta*- substituted phenyl analogues. Compound **IV-4** was identified as a potent (IC_{50} = 2.2 nM) and selective (versus CYP11B2, CYP17, and CYP19) CYP11B1 inhibitor. Since this compound also showed potent inhibition of rat CYP11B1 (facilitating in vivo test), it is considered to be a promising lead compound for further modification.

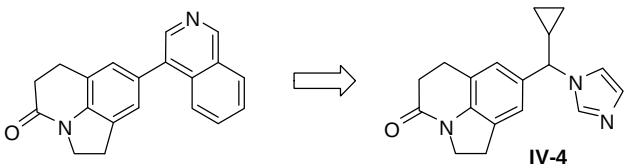

Figure 18. CYP11B1 inhibitor **IV-4** was designed.

Furthermore, it is interesting to observe that the simple exchang of N containing heterocycles (Figure 19), e.g. imidazolyl, 3-pyridyl or 4-pyridyl, dramatically changed the tendency of inhibiton. 4-Pyridyl substituted on the CH bridge resulted in CYP11B2 inhibitors, whereas imdazolyl and 3-pyridyl led to CYP11B1 inhibitors. This finding further demonstrated that a minor group could be the key to selectivity among CYP enzymes.

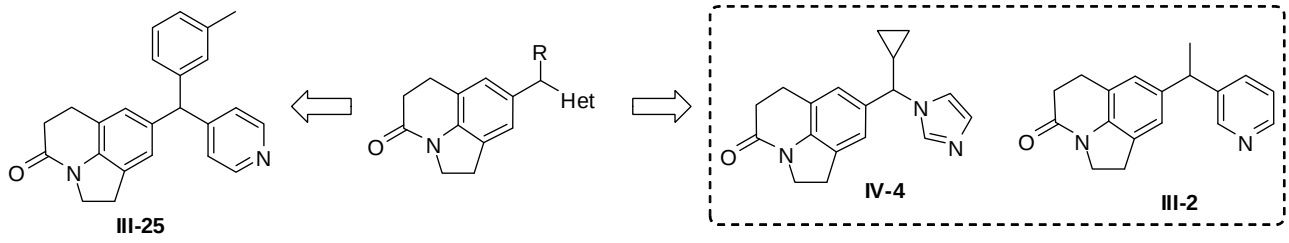

Figure 19. Different heterocycles on the CH bridge.

In conclusion, inhibition of CYP enzymes is a promising therapeutic strategy for treatment of severe hormone dependent diseases. In this study, compounds **II-11**, **II-20** as well as **IV-4** have been identified as potent and selective inhibitors of CYP11B2 and CYP11B1 inhibitor, respectively. Compound **III-25** is discovered to be a promising dual inhibitor of CYP19 / 11B2. These compounds can be considered as drug candidates of the further evaluation in vivo. Moreover, the SARs found in this study, such as CYP1A2 inhibition and minor changes of heterocycles alter the preference among CYP enzymes, could be valuable for further desigh of potent and selective inhibitors of various CYP enzymes.

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