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Identification of 4-(4-Nitro-2-phenethoxyphenyl)pyridine as a promising new Lead for Discovering Inhibitors of both Human and Rat 11β-Hydroxylase

Qingzhong Hu, a,b Jessica Kunde, a Nina Hanke, c and Rolf W. Hartmann a,b,*

a Pharmaceutical and Medicinal Chemistry, Saarland University, Campus C2.3, D-66123 Saarbrücken, Germany

b Helmholtz Institute for Pharmaceutical Research Saarland (HIPS), Campus C2.3, D-66123 Saarbrücken, Germany

c Elexopharm GmbH, Campus A1, 66123 Saarbrücken, Germany

* Author to whom correspondence should be addressed: Rolf W. Hartmann, Pharmaceutical and Medicinal Chemistry, Saarland University, Campus C2 3, D-66123 Saarbrücken, Germany, Phone: +49 681 302 70300. Fax: +49 681 302 70308. E-mail: rolf.hartmann@helmholtz-hzi.de.
Abstract

The inhibition of 11β-hydroxylase is a promising strategy for the treatment of Cushing’s syndrome, in particular for the recurrent and subclinical cases. To achieve proof of concept in rats, efforts were paid to identify novel lead compounds inhibiting both human and rat CYP11B1. Modifications on a potent promiscuous inhibitor of hCYP11B1, hCYP11B2 and hCYP19 (compound IV) that exhibited moderate rCYP11B1 inhibition led to compound 8 as a new promising lead compound. Significant improvements compared to starting point IV were achieved regarding inhibitory potency against both human and rat CYP11B1 (IC\textsubscript{50} values of 2 and 163 nM, respectively) as well as selectivity over hCYP19 (IC\textsubscript{50} = 1900 nM). Accordingly, compound 8 was around 7- and 28- fold more potent than metyrapone regarding the inhibition of human and rat CYP11B1 and exhibited a comparable selectivity over hCYP11B2 (SF of 3.5 versus 4.9). With further optimizations on this new lead compound 8, drug candidates with satisfying profiles are expected to be discovered.

Abbreviations

CYP, cytochrome P450; hCYP11B1, steroid 11β-hydroxylase; hCYP11B2, aldosterone synthase; hCYP17, 17α-hydroxylase-17,20-lyase; hCYP19, aromatase; SF, selectivity factor = IC\textsubscript{50 \text{hCYP11B2}} / IC\textsubscript{50 \text{hCYP11B1}}.

Keywords: 11β-hydroxylase, human and rat CYP11B1, inhibitors, Cushing’s syndrome, selectivity, aromatase
1. Introduction

Although endogenous Cushing’s syndrome is a rare disease with an annual incidence as low as 2–3 cases per million of the population, [1] it leads to a high mortality via co-morbidities including cardio- and cerebrovascular diseases. [1,2] As for the majority of this syndrome that are caused by pituitary adenomas and termed Cushing’s disease, the 5 years’ survival is only about 50% if without effective treatments. [3] Despite of the fact that the surgical removal of tumors leads to cure or remission in 65–85% of patients, recurrences are observed in up to 20% of cases. [1] Furthermore, investigations indicate a high prevalence of undiagnosed subclinical Cushing’s syndrome in particular in patients with type II diabetes and osteoporosis. [4,5] Although no evident symptoms other than high levels of cortisol in plasma are observed in these patients, this syndrome is considered to exacerbate concomitant diseases. More severe is that subclinical Cushing's syndrome is deemed to be associated with metabolic dementia and to promote the progression of Alzheimer disease. [6] Apparently, for these recurrent and subclinical cases, pharmacotherapy via the inhibition of steroid 11β-hydroxylase (hCYP11B1), which catalyzes the hydroxylation of 11-deoxycortisol to cortisol, to reduce the circulating cortisol levels is a superior approach. Metyrapone (IC$_{50}$ = 15 nM, Chart 1) as an inhibitor of adrenal steroidogenesis has been employed to relieve patients’ symptoms before surgery; [7] and its long-term applications were also reported to successfully control cortisol levels and psychiatric manifestations. [8,9] In a phase I clinical study, an experimental hCYP11B1 inhibitor LCI699 (IC$_{50}$ = 2.9 nM, Chart 1) normalized urinary free cortisol levels or reduced its concentrations by more than half from baseline in patients with moderate-to-severe Cushing’s disease. [10] However, simultaneously, the plasma aldosterone levels were significantly decreased by around 3-fold from 4.2 to 1.3 ng/dL because of the potent inhibition of aldosterone synthase (hCYP11B2) by LCI699 (IC$_{50}$ = 0.2 nM). Although this inhibition contributed to the reduction of both systolic and diastolic blood pressures (10.0 and 6.0 mmHg, respectively), it, together with the twofold elevated
adrenocorticotropic hormone in plasma that is the response to the drop of cortisol levels, boosted the concentrations of its precursor 11-deoxycorticosterone by 42-fold from 3.5 to 147.5 ng/dL. The binding of these over-produced 11-deoxycorticosterone to mineralocorticoid receptors induced around 8% reduction of the plasma potassium levels and consequently hypokalemia in one-third (4 out of 12) of patients. As evidenced by the decreased plasma renin levels (13 to 8 ng/L), which remained suppressed two weeks after the treatment discontinuation, the renin–angiotensin–aldosterone system was impaired. Therefore, the selectivity over \( h\text{CYP11B2} \) is considered to be a crucial criterion for safety when treating Cushing’s syndrome via \( h\text{CYP11B1} \) inhibition. Besides this indication, topical application of \( h\text{CYP11B1} \) inhibitors has also been proposed as a novel strategy to promote the healing of chronic wounds [11] based on the observation that cutaneously over-expressed cortisol impairs fibroblast proliferation and collagen synthesis, and thus induces chronic wounds and ulcers. [12] Cortisol also stimulates the proliferation of prostate cancer cells via certain mutated androgen receptors, [13] therefore the combinatory application of \( h\text{CYP11B1} \) inhibitors [14] or dual inhibitors of 17α-hydroxylase-17,20-lyase (\( h\text{CYP17} \)) and \( h\text{CYP11B1} \) [15] could be superior approaches to improve the survival of prostate cancer patients. Since several other steroidogenic cytochrome P450 (CYP) enzymes including \( h\text{CYP11B2} \), \( h\text{CYP17} \) and aromatase (\( h\text{CYP19} \)) catalyze the biosynthesis of important steroidal hormones (mineralocorticoids, androgens and estrogens, respectively), drug candidates inhibiting \( h\text{CYP11B1} \) ought to show adequate selectivity over these enzymes, in particular \( h\text{CYP11B2} \) (as indicated by the results of the clinical trial of LCI699). The selectivity between \( h\text{CYP11B1} \) and \( h\text{CYP11B2} \) is especially challenging to achieve because of the high homology (> 93%) between these two enzymes. Despite of this difficulty, potent and selective inhibitors of \( h\text{CYP11B2} \) [16–21] were discovered recently. Aided by our broad experience in developing selective inhibitors of steroidogenic CYP enzymes, including \( h\text{CYP11B2} \), [22–26] \( h\text{CYP17} \) [27–33] and \( h\text{CYP19} \), [34–38] several classes of potent \( h\text{CYP11B1} \) inhibitors were
identified. [39–41] Examples like compounds I (IC₅₀ = 107 nM) [45] and II (IC₅₀ = 2 nM) [41] exhibited both potent inhibition of hCYP11B1 (Chart 1 & Table 1) and promising selectivity over other CYP enzymes (for hCYP11B2, selectivity factors (SF = IC₅₀ hCYP11B2 / IC₅₀ hCYP11B1) around 15 were observed). However, these compounds showed no (I, IC₅₀ > 10000 nM) to low (II, IC₅₀ = 2440 nM) inhibition toward rat CYP11B1 (Table 1), which makes it difficult to prove the concept in rats. Therefore, discovery of compounds with novel structures inhibiting both human and rat CYP11B1 is necessary and urgent. In this study, we describe our efforts to identify new lead compounds exploiting previously acquired knowledge on developing selective inhibitors of steroidogenic CYP enzymes.

2. Results

2.1. Design Concept

In our previous investigation of pyridyl substituted 3,4-dihydroquinolin-2(1H)-ones as potent inhibitors of hCYP11B2, an interesting SAR was observed that the bulkiness augment of the alkoxy substituent at the 7-position largely increased the hCYP11B1 inhibition. [42] The benzyloxy compound IV exhibited a 56-fold stronger hCYP11B1 inhibition than the corresponding methoxy analogue III (IC₅₀ = 2487 nM) with an IC₅₀ value of 44 nM (Chart 1). Although the inhibition of hCYP11B2 was accordingly enhanced to 22 nM thus only showing an inadequate selectivity factor of 0.5 for hCYP11B1, a trend of reverse preference between hCYP11B1 and hCYP11B2 inhibition was anticipated when comparing it to that of compound III (SF = 0.1). More important is that compound IV showed a moderate inhibition of rCYP11B1 (IC₅₀ = 1500 nM), which is apparently superior to the clinical used drug metyrapone (IC₅₀ = 4607 nM) and the previously identified potent hCYP11B1 inhibitors I (IC₅₀ > 10000 nM) and II (IC₅₀ = 2440 nM) (Chart 1 & Table 1). Due to the difficulty of identifying inhibitors of both human and rat CYP11B1 to facilitate the proof of concept in rats, compound IV was considered as a promising starting point for further modifications. However, as compound IV actually originated from a series of dual inhibitors of hCYP11B2
and hCYP19, [42,43] it showed significant inhibition of hCYP19 as well (IC$_{50}$ = 35 nM). Therefore, besides elevating the potencies against human and rat CYP11B1, improvement of the selectivity over both hCYP11B2 and hCYP19 is another challenge encountered. To solve these problems, modifications were performed as shown in Chart 1. Since the 3,4-dihydroquinolin-2(1H)-one core was regarded as a privileged structure for hCYP11B2 inhibition, [44] the lactam ring was opened and simplified into a phenyl substituted by various groups. As the insertion of a nitrogen atom into the core was demonstrated to be beneficial for the selectivity over hCYP11B2, [45] a pyridyl was also attempted replacing the benzene core. Based on the comparison between compounds III and IV, the alkoxyl moiety was speculated to be a key for both potency and selectivity. The influences of the chain length and the substituting position of the alkoxyl group were therefore investigated. Since such kind of inhibitors competitively inhibit CYP enzymes via the coordination between their $sp^2$ hybrid N and the heme iron, the convenience to form these interactions, which largely depend on the relative position of the N with regard to the hydrophobic core, would exhibit significant impacts on both potency and selectivity. This factor was therefore examined by introducing either 3- or 4-pyridyl moieties as the heme-coordinating heterocycles as well as by inserting a methylene moiety between the pyridyl ring and the core, which have been found to alter the inhibitory pattern among steroidogenic CYP enzymes. [11,39,43] These efforts led to compounds 1–25 that were subsequently evaluated for their inhibition of both human and rat CYP11B1 as well as the selectivity over hCYP11B2, hCYP17 and hCYP19.

2.2. Chemistry

The syntheses of the designed compounds are shown in schemes 1 to 3. For the majority of desired compounds, a straightforward general route was employed (Scheme 1). The starting bromo phenols that were substituted with various groups, such as NO$_2$, F, OMe, CF$_3$, CN and CONMe$_2$, were either commercially available or obtained from cleavage of the corresponding methoxy precursors. They were firstly alkylated using the corresponding bromines to afford
ether intermediates 4a–6a, 10a, 12a, 14a, 16a, 18a, 20a and 22a before the 3- or 4-pyridyl moieties were introduced via Suzuki coupling reaction resulting in final compounds 4–23. This reaction sequence was established after having observed low yields of alkylation with pyridyl substituted phenols. In most cases, the ordinary Suzuki coupling condition, i.e. boiling the corresponding reactants with Pd(PPh₃)₄ and Na₂CO₃ in a mixture of ethanol, toluene and water, gave satisfying yields; while, for compounds 10–13, 18, 19 and 23, PdCl₂(dppf) had to be employed as an alternative. The nitro group in compound 4 was reduced to amino (3) using hydrazine hydrate and Pd/C, which was further acylated with acetyl chloride or 4-fluorobenzoyl chloride to obtain final compounds 1 and 2, respectively. To achieve the introduction of a methylene moiety between the pyridyl and the benzene core, the bromo group in 5a was first converted into borate (24a) using bis(pinacolato) diboron under the catalysis of PdCl₂(PPh₃)₂, which subsequently coupled with 3- or 4-(bromomethyl)pyridine to yield compounds 24 and 25, respectively.

2.3. Biological Results and Discussions

2.3.1. Inhibition of hCYP11B1 and hCYP11B2

The synthesized compounds 1–25 were tested for their inhibition of hCYP11B1 and hCYP11B2 in V79MZ cells expressing the corresponding enzymes, respectively, with [³H]-11-deoxycorticosterone as substrate. [46] Results are presented in Table 1 with metyrapone and compound I–IV as references.

The 3,4-dihydroquinolin-2(1H)-one core in the lead compound IV was opened as the first step of modification because this structure was regarded as a privileged structure for hCYP11B2 inhibition. [44] In contrast, the 3-pyridyl was left untouched to ensure direct comparisons of inhibitory potencies. The lactam ring was simplified into an acetamido group substituting the benzene core to maintain potential hydrogen bonds formed by the amide group. As expected, the resulting compound 1 exhibited preference for hCYP11B1 inhibition with a SF of 2.3, which is around 5-fold better than that of the lead compound IV. However, its hCYP11B1
inhibition was simultaneously reduced to 279 nM. A bulky 4-fluorobenzamido group (2) further decreased the inhibitory potency by 4-fold to 1207 nM. This observation is probably a result of steric clashes caused by increased bulkiness and/or rotation freedom after removing the astriction of the lactam ring. The replacement of amido groups by an amino moiety (3) only led to a moderate inhibition of hCYP11B1 (IC$_{50}$ = 540 nM). Surprisingly, the nitro analogue 4 showed potent hCYP11B1 inhibition with an IC$_{50}$ value of 23 nM, which is twice as potent as the lead IV (IC$_{50}$ = 44 nM). However, compound 4 failed to exhibit any superiority to lead IV regarding selectivity over hCYP11B2 (SF = 0.7). With the nitro group sustained, the influence of the chain length of the alkoxyl moiety was subsequently investigated. As the augmentation of methylene units, the inhibition of hCYP11B1 was elevated from 23 nM (4, n = 0, benzoxy) to 13 nM (5, n = 1) and to 6 nM (6, n = 2). Despite of the fact that all compounds also showed potent inhibition of hCYP11B2, improvements of selectivity compared to lead compound IV (SF = 0.5) was achieved, in particular for compound 5, which exhibited a SF of 4.1. The displacement of 3-pyridyl by 4-pyridyl did not influence much the inhibitory potency of hCYP11B1. The corresponding benzoxy compound 7 was also a potent inhibitor with an IC$_{50}$ value of 50 nM. With the insertion of more methylene units, a similar increase of inhibitory potency was observed. Phenethoxy analogue 8 (n = 1, IC$_{50}$ = 2 nM) was 25-fold more potent than the benzoxy compound 7; while an IC$_{50}$ of 8 nM was observed for the phenylpropoxy compound 9. Being the most potent hCYP11B1 inhibitor in this study, compound 8 was 22- and 7-fold more potent than the lead compound IV and metyrapone, respectively, and also exhibited a similar selectivity over hCYP11B2 as metyrapone. The fact that both 3- and 4-pyridyl compounds showed similar potency is an indication of a relative large pocket, in which the benzene cycle could drift a bit to provide the suitable angle to form the N-Fe coordination. The high flexibility of the alkoxyl side chain could enable the compounds to adapt to the angle change. In contrast, the position of the alkoxyl group was obviously more decisive for hCYP11B1 inhibition. The shift from the
ortho- (with regard to the pyridyl) to the meta-position resulted in decreases of potency by 85- and 600-fold for the 3- and 4-pyridyl analogues (10, IC\textsubscript{50} = 508 nM; and 11, IC\textsubscript{50} = 1201 nM), respectively. Since phenethoxy analogues showed better selectivity (SF of 4.1 for compound 5 and 3.5 for compound 8), this chain length was sustained in the subsequent modifications. In contrast, both 3- and 4-pyridyl analogues were synthesized to avoid missing any potential strong inhibitors. Although nitro groups present in a considerable number of drugs in clinical use, such as nifedipine, they have the potential of being reduced to aromatic nitrosos, which can covalently binds to DNA and proteins, and thus are carcinogenic. [47] Wariness was therefore put on the nitro groups and the replacement of nitro by other substituents was attempted. When relatively small groups were introduced into the molecules (F in 12 & 13; OMe in 15; CF\textsubscript{3} in 17; and CN in 18 & 19), potent hCYP11B1 inhibition was observed with IC\textsubscript{50} values less than 60 nM. CN in combination with 4-pyridyl led to the most potent compound in this small set with an IC\textsubscript{50} value of 12 nM. An exception is OMe in the 3-pyridyl compound 14, which showed a moderate hCYP11B1 inhibition (IC\textsubscript{50} = 183 nM); whereas CF\textsubscript{3} in combination with 3-pyridyl surprisingly only resulted in a weak inhibitory potency (IC\textsubscript{50} = 812 nM). Accordingly, the bulkier CONMe\textsubscript{2} substituents led to weak inhibition with IC\textsubscript{50} values of 594 and 1150 nM for 3-Py (20) and 4-Py (21) compounds, respectively, indicating potential steric hindrances. In contrast to bulkiness, other properties, such as electrostatic potential and the ability to form hydrogen bonds, seem to play only minor roles. When the substituent is as small as F, no significant difference in potency was found between 3- and 4-pyridyl compounds 12 and 13 (IC\textsubscript{50} values of 51 and 60 nM, respectively); while for other groups with larger size (OMe, CF\textsubscript{3} and CN in compounds 14–19), the 4-pyridyl analogues were significantly more potent than the corresponding 3-pyridyl compounds. Although more potent hCYP11B1 inhibitors were identified via these replacements, no improvement of selectivity over hCYP11B2 was observed (SFs < 2). As the replacement of the benzene core by a pyridyl moiety in the biaryl methylene substituted heterocycle class of hCYP11B1
inhibitors increased the selectivity over hCYP11B2, [45] a nitrogen atom was inserted into the benzene core leading to compounds 22 and 23. However, this modification strongly reduced the hCYP11B1 inhibition (IC$_{50}$ > 1400 nM). Moreover, the introduction of a methylene moiety between the benzene core and the 3-pyridyl group (24) decreased the potency by around 10-fold compared to the corresponding analogue 5; whereas the same modification on 4-pyridyl compound 25 led to a potent hCYP11B1 inhibitor with similar potency (IC$_{50}$ = 6 nM) as the parent compound 8.

2.3.2. Inhibition of human CYP17 and CYP19

Since this series of compounds were originated from the lead compound IV, which showed a potent inhibition of hCYP19 (IC$_{50}$ = 35 nM), the improvement of the corresponding selectivity was a tough task throughout the modifications. The most potent hCYP11B1 inhibitors 4–9, 17–9, 24 and 25 were therefore evaluated for their inhibition of hCYP19 with human placental microsomes using [1β-^3^H]androstenedione (500 nM) as a substrate. [48] Apparently, all 3-pyridyl compounds (4–6, 18 and 24) exhibited potent inhibition of hCYP19 with IC$_{50}$ values below 170 nM (Table 1). In contrast, 4-pyridyl analogues (7–9, 17 and 29) showed very weak to no inhibition toward hCYP19 with IC$_{50}$ values ranging from 1500 to 5000 nM. The only exception was compound 25 (IC$_{50}$ = 157 nM) probably due to the methylene moiety between the heme-coordinating pyridyl and the benzene core. With an improvement of more than 40-fold on hCYP19 selectivity compared to lead IV, these 4-pyridyl compounds are not likely to reduce estrogen biosynthesis under the treatment doses when taking into consideration their very strong inhibitory potency against the target enzyme hCYP11B1.

Since hCYP17 is of crucial importance for the production of androgens, the selectivity over this steroidogenic enzyme was employed as another criterion for safety. The above-mentioned compounds were further investigated for their hCYP17 inhibition using the 50,000 g sediment of an E. coli homogenate recombinantly expressing hCYP17 with progesterone (25 µM) as a
substrate. [49] No inhibition (IC₅₀ > 10 µM) was observed for all compounds tested indicating safety with regard to this aspect.

2.3.3. Inhibition of rat CYP11B1

As one of the major tasks of this study, the inhibition of rCYP11B1 was determined for the most potent inhibitors of the human enzyme (same as mentioned in the last section) in V79MZ cells expressing rCYP11B2. [50] Interestingly, for nitro compounds, all 4-pyridyl analogues 7–9 (IC₅₀ < 650 nM) were much stronger than 3-pyridyl compounds 4–6 (IC₅₀ ranging from 1300 to 2000 nM). Compound 8 as the most potent one exhibited an IC₅₀ value of 163 nM, which was near 10- and 28-fold stronger than lead IV and metyrapone, respectively. In contrast, when substituted with CN, no significant difference in potency between 3- and 4-pyridyl compounds 18 and 19 was observed (IC₅₀ around 500 nM); whereas, in the case of methylene compounds, the 4-pyridyl derivative 25 showed an about 4-fold weaker inhibition compared to the corresponding 3-pyridyl compound 24 (IC₅₀ of 2850 versus 706 nM), which is in contrast to the SAR observed with the nitro compounds.

2.3.4. Modelling studies

To further investigate the interactions between the synthesized inhibitors and the targeting enzyme, a homology model of human CYP11B1 was established using the reported crystal structure of hCYP11B2 as the template. The sequences of both hCYP11B1 (UniProt code: P15538) and hCYP11B2 (UniProt code: P19099) comprise 503 amino acids, among which 473 are identical (Figure S1). In the crystal of hCYP11B2 in complex with deoxycorticosterone (PDB ID: 4DVQ, chain A), amino acids 34-502 present. Since amino acids 1-24 as a transit peptide is less relevant to biological functions and configuration, amino acids 1-33 in the hCYP11B1 sequence was omitted similar as in the template crystal when establishing the model. A library of ten models was initially generated and the best one was selected according to electrostatic solvation energy and root mean square deviation of each intermediate model to the average position of all models. This preliminary model was
subsequently further refined via molecular dynamic simulation to yield the final one. The quality of the resulting homology model was evaluated regarding the stereochemical measurements of each amino acid including bond lengths, angles, dihedrals and φ-ψ torsions. As shown in the Ramachandran scatter plot (Figure S2), 451 out of 470 amino acid (96 %) clustered in favorable regions, whereas only one amino acid (Ser288) was considered as an outlier (0.2 %). This hCYP11B1 homology model presented characteristic cytochrome P450 folds and nicely superposed to the template structure (Figure 1A). Although 30 amino acids differ in the sequences of these two enzymes, they locate outside the active sites. It is therefore not surprising that the configuration of their active sites are very similar. However, a scrutiny on the active sites still revealed minor differences (Figures 1B), which could be the basis and keys for inhibitors to selectively inhibit an individual enzyme. In the hCYP11B1 homology model, the middle part of the I-helix extruded slightly toward the opposite β2-sheet compared to that in the hCYP11B2 crystal thus narrowing the binding pocket; while the F-helix and the heme moiety as the roof and the floor of the active site, respectively, subsided by around 2 Å synchronously. Although drifts were also observed for the β2-sheet, the B-B’ loop and the (β4-1)-(β4-2) loop, alterations were identified in the B’-C loop that are subtle nevertheless of greater importance in changing the binding site (Figure 1C). The side chain of Leu131 jostled toward the β2-sheet thus shrinking the hydrophobic pocket in between. In contrast, the phenyl group of Phe130 turned downward to heme moiety leading to more convenience for inhibitors to enter and exploit this pocket. The most promising compound (8) in this study was subsequently docked into the hCYP11B1 homology model to investigate the interactions between inhibitors and targeting enzyme. The molecule was anchored by the coordination between the $sp^2$ hybrid N in pyridyl and the heme iron and leaned on the β2-sheet with an angle of approximate 60° with respect to the heme plane (Figure 2A). The phenethoxy group stretched out pointing to the B-B’ loop and fitted into the hydrophobic pocket mentioned above, which was delimited by Arg110-Met111-Ser112-Leu113-Leu131-
Glu383 (Figure 2B). The phenyl side chains of Phe130 and Phe381 served as two gates of this pocket and formed π-π interactions with the phenethoxyl group in parallel and perpendicular manners, respectively. An arene-cation interaction was also observed between the phenethoxyl substituent and the guanidyl of Arg110. Since the side chains of Glu383 and Met111 were accessible, the introduction of positive-charged groups and/or hydrogen bond donors onto the phenethoxyl moiety to form salt bridge and/or hydrogen bond would elevate inhibitory potency. As this pocket in hCYP11B1 homology model exhibited a more convenient entrance (Phe130) but a reduced volume (Leu131) compared to that in the hCYP11B2 crystal structure, this feature could be exploited to achieve more selectivity. Furthermore, Phe130 and Phe381 formed π-π interactions with the pyridyl moiety (parallel) and the phenyl core (perpendicular) and thus promoted affinity and stabilized the binding pose. An additional π-π interaction between Phe487 and the phenyl core (parallel) as well as hydrogen bonds formed by the nitro group with Tyr485 and Leu380 also contributed significantly with this regard. The drift of I-helix toward the β2-sheet narrowing the active site possibly facilitated the formation of these hydrogen bonds, but also increased the risks of steric clashes for bulkier substituents, which is in accordance with the SAR discussed above.

3. Conclusions

As evidenced by the clinical application of metyrapone and the phase I clinical study of LCI699, the inhibition of hCYP11B1 is an elegant approach to treat Cushing’s syndrome, in particular for the recurrent and subclinical cases. To overcome the inconvenience in achieving proof of concept in rats, which is caused by the impotency of our previously discovered hCYP11B1 inhibitors against the corresponding rat enzyme, efforts were paid to identify novel lead compounds with different structures. Modifications on a potent promiscuous inhibitor (IV) of hCYP11B1, hCYP11B2 and hCYP19 (IC\textsubscript{50} values of 44, 22 and 35 nM, respectively) that exhibited moderate rCYP11B1 inhibition (IC\textsubscript{50} = 1500 nM) led to compound 8 as a new promising lead compound. Compared to the starting point compound
IV, this inhibitor showed not only 22- and 9-fold elevations on both human and rat CYP11B1 inhibition with IC$_{50}$ values of 2 and 163 nM, respectively, but also significant improvement of selectivity over hCYP19 (54-fold, IC$_{50} =$ 1900 nM). Accordingly, compound 8 was around 7- and 28- fold more potent than the drug metyrapone regarding the inhibition of human and rat CYP11B1. Although its selectivity over hCYP11B2 was also increased by a factor of 7 (SF of 3.5 versus 0.5) compared to compound IV reaching a similar level as metyrapone (SF = 4.9), it was considered insufficient and remains to be improved. With further optimizations on the new lead compound 8 that was successfully identified in this study, drug candidates with satisfying profiles are expected to be discovered for the consequent drug development and the research of pathophysiology involving CYP11B1.

4. Experimental section

4.1. Chemistry

4.1.1. Chemical and Analytical Methods

Melting points were measured on a Mettler FP1 melting point apparatus and are uncorrected. $^1$H-NMR and $^{13}$C-NMR were recorded on a Bruker AM500 spectrometer at 500 MHz and 125 MHz, respectively, at 300 K. Chemical shifts are reported in parts per million (ppm), by reference to the hydrogenated residues of the deuterated solvent as internal standard. All coupling constants ($J$) are given in Hertz (Hz). Mass spectra (LC/UV/MS: ESI) were recorded on a SpectraSystem/MSQ Plus (ThermoFinnigan) instrument with a RP18-100-5 column (Macherey-Nagel). A water/acetonitrile gradient was used as eluent system. All compounds are > 95 % chemical pure as measured by LC/UV trace at 254 nm. Reagents were used as obtained from commercial suppliers without further purification. Solvents were distilled before use. If necessary, solvents were dried by distillation from appropriate drying reagents prior to use. Flash chromatography was performed on silica gel 40 (35/40–63/70 µM) with petroleum ether/ethyl acetate or CH$_2$Cl$_2$/methanol mixtures as eluents. Reaction progress was
monitored by thin-layer chromatography (TLC) on TLC Silica Gel 60 F254 (Merck KGaA). Visualization was accomplished with UV light.

4.1.2. General procedure A: Methoxyl cleavage with boron tribromide

A solution of the corresponding methoxyl starting material in DCM (4 ml/mmol) was cooled to -20°C before boron tribromide (3 eq, 1 M in DCM) was added in a nitrogen atmosphere at the same temperature. The stirred solution was subsequently warmed to ambient temperature overnight. The resulted reaction mixture was diluted with water and the phases were separated. The aqueous layer was extracted three times with ethyl acetate and the combined organic layers were dried over Na₂SO₄. The solvent was removed under reduced pressure and the crude product was purified by flash chromatography on silica gel.

4.1.3. General procedure B: Etherization

To the corresponding bromophenol in ethanol (5 ml/mmol) was added K₂CO₃ (1.2 eq). The resulted mixture was stirred for 2 h under reflux conditions (90 °C) before the corresponding bromide (1.3 eq) and KI (5 mol%) were added. Reflux was continued overnight. After cooling down, the solvent was removed under reduced pressure and the residue was washed with brine and extracted three times with ethyl acetate. The combined organic layers were dried over Na₂SO₄, the solvent was removed under reduced pressure and the crude product was purified by flash chromatography on silica gel.

4.1.4. General procedure C: Suzuki coupling catalyzed by Pd(PPh₃)₄

The corresponding bromide (1.0 eq) and pyridylboronic acid (1.5 eq) were dissolved in a mixture of ethanol (10 ml/100 mg), toluene (10 ml/100 mg) and an aqueous 2.0 M Na₂CO₃ solution (2.5 ml/100 mg). The mixture was deoxygenated and flushed with nitrogen three times before Pd(PPh₃)₄ (5 mol%) was added. The resulting suspension was heated under reflux for 3-5 h. After cooling down, water and ethyl acetate were added and the phases were separated. The water phase was extracted three times with ethyl acetate and the combined
organic layers were dried over Na₂SO₄. The solvent was removed under reduced pressure and the crude product was purified by flash chromatography on silica gel.

4.1.5. General procedure D: Suzuki coupling catalyzed by PdCl₂(dppf)

The corresponding bromide (1.0 eq) and pyridylboronic acid (1.5 eq) were dissolved in a mixture of DME (10 ml/100mg) and an aqueous 2.0 M Na₂CO₃ solution (2.5 ml/100mg). The mixture was deoxygenated and flushed with nitrogen three times before PdCl₂(dppf) (5 mol%) was added. The resulting suspension was heated under reflux for 3-5 h or overnight. After cooling down, water and ethyl acetate were added and the phases were separated. The water phase was extracted three times with ethyl acetate and the combined organic layers were dried over Na₂SO₄. The solvent was removed under reduced pressure and the crude product was purified by flash chromatography on silica gel.

4.1.6. \( N\)-(3-(Benzyloxy)-4-(pyridin-3-yl)phenyl)acetamide (1)

To the solution of 3-(benzyloxy)-4-(pyridin-3-yl)aniline 3 (100 mg, 0.36 mmol), triethylamine (0.10 mL, 0.72 mmol) and \( N\),\( N\)-dimethylpyridin-4-amine (2 mg) in dichloromethane (20 mL) was dropped in acetyl chloride (29.8 mg, 0.38 mmol) in an ice bath. The resulting reaction mixture was subsequently stirred at room temperature overnight before water (20 mL) was added and the phases were separated. The water phase was extracted three times with dichloromethane (10 mL) and the combined organic layers were dried over Na₂SO₄. The solvent was removed under reduced pressure and the crude product was purified by flash chromatography on silica gel using a mixture of dichloromethane / methanol (50:1) as eluent. Yield: 109 mg (95%); yellow solid: mp 157–159 °C; \( R_f \) = 0.29 (n-Hex/ EtOAc 1:2); Anal. C20H18N2O2 (C, H, N, O). \(^1\)H-NMR (CDCl₃, 500 MHz): \( \delta_H = 8.78 \) (d, \( J = 1.7 \) Hz, 1H), 8.54 (dd, \( J = 1.7, 4.8 \) Hz, 1H), 7.87–7.89 (m, 1H), 7.47 (d, \( J = 1.7 \) Hz, 1H), 7.37 (s, br, 1H), 7.31–7.34 (m, 1H), 7.24–7.27 (m, 3H), 7.15–7.18 (m, 1H), 7.10–7.12 (m, 2H), 6.97 (dd, \( J = 1.9, 8.2 \) Hz, 1H), 4.79 (s, 2H), 2.20 (s, 3H); \(^13\)C-NMR (CDCl₃, 125
MHz): $\delta_C = 156.4, 150.1, 147.7, 141.3, 139.3, 136.8, 136.7, 133.9, 130.6, 128.4, 125.9, 123.0, 122.8, 111.7, 104.3, 70.4, 20.5$; MS (ESI): $m/z = 319.14 [M+H]^+$. 

4.1.7. **N-(3-(Benzyloxy)-4-(pyridin-3-yl)phenyl)-4-fluorobenzamide (2)**

To the solution of 3-(benzyloxy)-4-(pyridin-3-yl)aniline 3 (100 mg, 0.36 mmol), triethylamine (0.10 mL, 0.72 mmol) and $N,N$-dimethylpyridin-4-amine (2 mg) in dichloromethane (20 mL) was dropped in 4-fluorobenzoyl chloride (60.0 mg, 0.38 mmol) in an ice bath. The resulting reaction mixture was subsequently stirred at room temperature overnight before water (20 mL) was added and the phases were separated. The water phase was extracted three times with dichloromethane (10 mL) and the combined organic layers were dried over Na$_2$SO$_4$. The solvent was removed under reduced pressure and the crude product was purified by flash chromatography on silica gel using a mixture of dichloromethane / methanol (50:1) as eluent. Yield: 130 mg (91%); yellow solid: mp 166–167 °C; $R_f = 0.35$ (n-Hex/EtOAc 1:2); Anal. C$_{25}$H$_{19}$FN$_2$O$_2$ (C, H, N, O). $^1$H-NMR (CDCl$_3$, 500 MHz): $\delta_H = 8.82$ (d, $J = 1.7$ Hz, 1H), 8.63 (dd, $J = 1.7, 4.8$ Hz, 1H), 8.10 (dd, $J = 1.9, 8.2$ Hz, 2H), 7.92–7.94 (m, 1H), 7.65 (d, $J = 1.7$ Hz, 1H), 7.59 (s, br, 1H), 7.24–7.35 (m, 6H), 7.17–7.22 (m, 3H), 6.95 (m, 1H), 4.76 (s, 2H); $^{13}$C-NMR (CDCl$_3$, 125 MHz): $\delta_C = 159.7$ (d, $J_{CF} = 245.7$ Hz), 154.7, 147.3, 138.8, 137.9, 134.7, 126.9, 123.4, 119.8 (d, $J_{CF} = 10.1$ Hz), 119.7 (d, $J_{CF} = 10.6$ Hz), 119.2, 118.9, 118.7, 117.2, 117.1, 114.0, 111.1, 105.8 (d, $J_{CF} = 26.6$ Hz), 105.6 (d, $J_{CF} = 26.1$ Hz), 103.7, 102.0, 71.5; MS (ESI): $m/z = 399.14 [M+H]^+$. 

4.1.8. **3-(Benzyloxy)-4-(pyridin-3-yl)aniline (3)**

To the suspension of 3-(2-(benzyloxy)-4-nitrophenyl)pyridine 4 (25 mg, 0.08 mmol) and Pd/C (2.2 mg, 10% mmol) in ethanol (5 mL) was added hydrazine hydrate (11.2 mg, 0.25 mmol) slowly in an ice bath. The resulting reaction mixture was subsequently heated to reflux for 1 h. After cooling down to room temperature, water (10 mL) and ethyl acetate (10 mL) were added and the phases were separated. The water phase was extracted three times with ethyl acetate (10 mL) and the combined organic layers were dried over Na$_2$SO$_4$. The solvent
was removed under reduced pressure and the crude product was purified by flash chromatography on silica gel using a mixture of dichloromethane / methanol (50:1) as eluent. Yield: 19 mg (86%); yellow solid: mp 172–174 °C; \( R_f = 0.21 \) (n-Hex/EtOAc 1:2); Anal. C18H16N2O (C, H, N, O). \(^1\)H-NMR (CDCl\(_3\), 500 MHz): \( \delta_H = 8.67 \) (d, \( J = 2.6 \) Hz, 1H), 8.47 (dd, \( J = 1.7, 4.8 \) Hz, 1H), 7.65–7.67 (m, 1H), 7.26–7.29 (m, 2H), 7.15–7.17 (m, 2H), 7.09 (d, \( J = 8.1 \) Hz, 1H), 6.35 (dd, \( J = 2.1, 8.1 \) Hz, 1H), 6.30 (d, \( J = 2.1 \) Hz, 1H), 4.79 (s, 2H), 3.85 (s, br, 2H, NH\(_2\)); \(^1^3\)C-NMR (CDCl\(_3\), 125 MHz): \( \delta_C = 156.9, 150.0, 147.9, 146.9, 136.7, 134.4, 129.0, 128.4, 126.5, 122.6, 117.5, 107.6, 99.4, 70.4 \); MS (ESI): \( m/z = 277.13 \) [M+H]+.

4.1.9. 3-(2-(Benzyloxy)-4-nitrophenyl)pyridine (4)
The title compound was synthesized from 2-(benzyloxy)-1-bromo-4-nitrobenzene 4a (108 mg, 0.35 mmol) according to method C using 3-pyridylboronic acid. The crude product was purified by flash chromatography on silica gel using a mixture of petroleum ether / ethyl acetate (2:1) as eluent. Yield: 80 mg (75%); lemon yellow solid: mp 121–123 °C; \( R_f = 0.37 \) (n-Hex/EtOAc 1:2); Anal. C18H14N2O3 (C, H, N, O). \(^1\)H-NMR (CDCl\(_3\), 500 MHz): \( \delta_H = 8.71 \) (d, \( J = 1.9 \) Hz, 1H), 8.53 (dd, \( J = 1.4, 4.8 \) Hz, 1H), 7.85–7.88 (m, 2H), 7.79–7.81 (m, 1H), 7.40 (d, \( J = 8.2 \) Hz, 1H), 7.23–7.28 (m, 6H), 5.12 (s, 2H); \(^1^3\)C-NMR (CDCl\(_3\), 125 MHz): \( \delta_C = 156.0, 150.0, 149.3, 148.6, 136.8, 135.3, 134.2, 132.2, 131.0, 128.8, 128.4, 127.1, 123.0, 116.5, 107.9, 71.1 \); MS (ESI): \( m/z = 307.10 \) [M+H]+.

4.1.10. 3-(4-Nitro-2-phenethoxyphenyl)pyridine (5)
The title compound was synthesized from 1-bromo-4-nitro-2-phenethoxybenzene 5a (175 mg, 0.54 mmol) according to method C using 3-pyridylboronic acid. The crude product was purified by flash chromatography on silica gel using a mixture of petroleum ether / ethyl acetate (2:1) as eluent. Yield: 137 mg (78%); yellow solid: mp 125–126 °C; \( R_f = 0.39 \) (n-Hex/EtOAc 1:2); Anal. C19H16N2O3 (C, H, N, O). \(^1\)H-NMR (CDCl\(_3\), 500 MHz): \( \delta_H = 8.77 \) (s, 1H), 8.72 (d, \( J = 4.8 \) Hz, 1H), 8.00 (d, \( J = 8.3 \) Hz, 1H), 7.90 (s, 1H), 7.75 (d, \( J = 7.9 \) Hz, 1H), 7.52 (d, \( J = 8.3 \) Hz, 1H), 7.31–7.41 (m, 4H), 7.23 (d, \( J = 7.4 \) Hz, 2H), 4.40 (t, \( J = 6.5 \) Hz,
2H), 3.15 (t, \(J = 6.5\) Hz, 2H); \(^{13}\)C-NMR (CDCl\(_3\), 125 MHz): \(\delta_C = 156.3, 149.9, 149.2, 148.5, 137.6, 136.9, 133.8, 132.2, 130.9, 129.0, 128.5, 126.7, 122.9, 116.1, 107.0, 69.9, 35.4\); MS (ESI): \(m/z = 321.12\) [M+H]⁺.

4.1.11. 3-(4-Nitro-2-(3-phenylpropoxy)phenyl)pyridine (6)

The title compound was synthesized from 1-bromo-4-nitro-2-phenethoxybenzene 6a (125 mg, 0.37 mmol) according to method C using 3-pyridylboronic acid. The crude product was purified by flash chromatography on silica gel using a mixture of petroleum ether / ethyl acetate (2:1) as eluent. Yield: 89 mg (72%); yellow solid: mp 117–119 °C; \(R_f = 0.37\) (n-Hex/EtOAc 1:2); Anal. C\(_{20}\)H\(_{18}\)N\(_2\)O\(_3\) (C, H, N, O). \(^1\)H-NMR (CDCl\(_3\), 500 MHz): \(\delta_H = 8.79\) (d, \(J = 41.5\) Hz, 1H), 8.62 (dd, \(J = 1.5, 4.8\) Hz, 1H), 7.92 (dd, \(J = 2.1, 8.4\) Hz, 1H), 7.87 (dt, \(J = 1.8, 7.9\) Hz, 1H), 7.77 (d, \(J = 2.1\) Hz, 1H), 7.45 (d, \(J = 8.3\) Hz, 1H), 7.36–7.39 (m, 1H), 7.23–7.25 (m, 2H), 7.15–7.18 (m, 2H), 7.08 (d, \(J = 7.0\) Hz, 2H), 4.07 (t, \(J = 6.2\) Hz, 2H), 2.68 (t, \(J = 7.3\) Hz, 2H), 2.06–2.09 (m, 2H); \(^{13}\)C-NMR (CDCl\(_3\), 125 MHz): \(\delta_C = 156.6, 150.3, 149.5, 148.9, 141.0, 137.0, 134.1, 132.6, 131.1, 128.8, 128.6, 126.4, 123.2, 116.4, 107.4, 68.4, 32.3, 30.7\); MS (ESI): \(m/z = 335.13\) [M+H]⁺.

4.1.12. 4-(2-(Benzyloxy)-4-nitrophenyl)pyridine (7)

The title compound was synthesized from 2-(benzyloxy)-1-bromo-4-nitrobenzene 4a (150 mg, 0.49 mmol) according to method C using 4-pyridylboronic acid. The crude product was purified by flash chromatography on silica gel using a mixture of petroleum ether / ethyl acetate (2:1) as eluent. Yield: 107 mg (72%); yellow solid: mp 129–121 °C; \(R_f = 0.33\) (n-Hex/EtOAc 1:2); Anal. C\(_{18}\)H\(_{14}\)N\(_2\)O\(_3\) (C, H, N, O). \(^1\)H-NMR (CDCl\(_3\), 500 MHz): \(\delta_H = 8.69\) (s, 2H), 7.94–7.96 (m, 2H), 7.47–7.51 (m, 2H), 7.33–7.37 (m, 5H), 5.12 (s, 2H); \(^{13}\)C-NMR (CDCl\(_3\), 125 MHz): \(\delta_C = 156.2, 150.1, 149.1, 144.3, 135.5, 134.9, 131.2, 129.0, 128.7, 127.4, 124.3, 115.7, 108.2, 71.4\); MS (ESI): \(m/z = 307.10\) [M+H]⁺.

4.1.13. 4-(4-Nitro-2-phenethoxyphenyl)pyridine (8)
The title compound was synthesized from 1-bromo-4-nitro-2-phenethoxybenzene 5a (150 mg, 0.47 mmol) according to method C using 4-pyridylboronic acid. The crude product was purified by flash chromatography on silica gel using a mixture of petroleum ether / ethyl acetate (2:1) as eluent. Yield: 104 mg (70%); yellow solid: mp 112–114 °C; \( R_t = 0.32 \) (n-Hex/EtOAc 1:2); Anal. C19H16N2O3 (C, H, N, O). \(^1^H\)-NMR (CDCl\(_3\), 500 MHz): \( \delta_H = 8.60 \) (dd, \( J = 1.6, 4.5 \) Hz, 2H), 7.87 (dd, \( J = 2.2, 8.4 \) Hz, 1H), 7.79 (d, \( J = 2.1 \) Hz, 1H), 7.40 (d, \( J = 8.4 \) Hz, 1H), 7.25–7.29 (m, 2H), 7.22–7.24 (m, 3H), 7.12–7.14 (m, 2H), 4.31 (t, \( J = 6.4 \) Hz, 2H), 3.04 (t, \( J = 6.5 \) Hz, 2H); \(^1^3^C\)-NMR (CDCl\(_3\), 125 MHz): \( \delta_C = 156.5, 150.0, 149.1, 144.2, 137.8, 134.5, 131.0, 129.2, 128.8, 127.0, 124.3, 116.3, 107.3, 70.1, 35.6; \) MS (ESI): \( m/z = 321.12 \) [M+H]\(^+\).

4.1.14. 4-(4-Nitro-2-(3-phenylpropoxy)phenyl)pyridine (9)

The title compound was synthesized from 1-bromo-4-nitro-2-phenethoxybenzene 6a (150 mg, 0.45 mmol) according to method C using 4-pyridylboronic acid. The crude product was purified by flash chromatography on silica gel using a mixture of petroleum ether / ethyl acetate (2:1) as eluent. Yield: 113 mg (76%); yellow solid: mp 127–199 °C; \( R_t = 0.32 \) (n-Hex/EtOAc 1:2); Anal. C20H18N2O3 (C, H, N, O). \(^1^H\)-NMR (CDCl\(_3\), 500 MHz): \( \delta_H = 8.71 \) (s, br, 2H), 7.91 (s, br, 1H), 7.79 (s, 1H), 7.45–7.47 (m, 3H), 7.23–7.27 (m, 2H), 7.15–7.19 (m, 1H), 7.10–7.12 (m, 2H), 4.09 (t, \( J = 6.2 \) Hz, 2H), 2.71 (t, \( J = 7.3 \) Hz, 2H), 2.06–2.10 (m, 2H); \(^1^3^C\)-NMR (CDCl\(_3\), 125 MHz): \( \delta_C = 156.3, 149.8, 148.9, 144.2, 140.7, 134.3, 130.8, 128.6, 128.3, 126.2, 124.0, 116.0, 107.2, 68.2, 32.1, 30.5; \) MS (ESI): \( m/z = 335.13 \) [M+H]\(^+\).

4.1.15. 3-(4-Nitro-3-phenethoxyphenyl)pyridine (10)

The title compound was synthesized from 4-bromo-1-nitro-2-phenethoxybenzene 10a (108 mg, 0.34 mmol) according to method D using 3-pyridylboronic acid. The crude product was purified by flash chromatography on silica gel using a mixture of petroleum ether / ethyl acetate (2:1) as eluent. Yield: 106 mg, (97 %); orange solid: mp = 91–92 °C; \( R_t = 0.36 \) (n-Hex/EtOAc 1:2); Anal. C19H16N2O3 (C, H, N, O). IR (ATR): \( \tilde{\nu} \) (cm\(^{-1}\)) = 3040 (m), 2940
(m), 1586 (s), 1505 (s), 1228 (s), 1022 (s), 734 (vs), 694 (vs); $^1$H-NMR (CDCl$_3$, 500 MHz): $\delta_H = 8.78$ (s, 1H), 8.63 (d, $J = 4.7$ Hz, 1H), 7.90 (d, $J = 8.5$ Hz, 1H), 7.88 (d, $J = 7.3$ Hz, 1H), 7.40–7.46 (m, 1H), 7.26–7.30 (m, 4H), 7.18–7.23 (m, 1H), 7.14 (dd, $J = 1.4$, 8.4 Hz, 1H), 7.11 (s, 1H), 4.32 (t, $J = 6.8$ Hz, 2H), 3.14 (t, $J = 6.8$ Hz, 2H); $^{13}$C-NMR (CDCl$_3$, 125 MHz); $\delta_C = 152.9$, 148.5, 147.0, 143.3, 139.5, 137.4, 135.8, 135.4, 129.2, 128.6, 126.8, 126.5, 124.2, 119.1, 113.2, 70.9, 35.7; MS (ESI): $m/z = 320.88$ [M+H]$^+$.  

4.1.16. 4-(4-Nitro-3-phenethoxyphenyl)pyridine (11)

The title compound was synthesized from 4-bromo-1-nitro-2-phenethoxybenzene 11a (139 mg, 0.43 mmol) according to method D using 4-pyridylboronic acid. The crude product was purified by flash chromatography on silica gel using a mixture of petroleum ether / ethyl acetate (1:1) as eluent. Yield: 129 mg (94%); ochre solid: mp = 98 °C; $R_f = 0.27$ (n-Hex/EtOAc 1:2); Anal. C19H16N2O3 (C, H, N, O). IR (ATR): $\tilde{\nu}$ (cm$^{-1}$) = 3032 (m), 2951 (m), 1586 (s), 1552 (s), 1509 (s), 1225 (s), 808 (vs), 755 (vs), 698 (vs); $^1$H-NMR (CDCl$_3$, 500 MHz): $\delta_H = 8.66$ (d, $J = 5.4$ Hz, 2H), 7.89 (d, $J = 8.5$ Hz, 1H), 7.44 (d, $J = 5.0$ Hz, 2H), 7.25–7.30 (m, 4H), 7.17–7.22 (m, 2H), 7.15 (d, $J = 1.6$ Hz, 1H), 4.32 (t, $J = 6.8$ Hz, 2H), 3.14 (t, $J = 6.8$ Hz, 2H); $^{13}$C-NMR (CDCl$_3$, 125 MHz); $\delta_C = 152.9$, 149.7, 147.2, 143.8, 140.0, 137.4, 129.2, 128.6, 126.9, 126.4, 122.0, 119.0, 113.2, 70.9, 35.6; MS (ESI): $m/z = 320.96$ [M+H]$^+$.  

4.1.17. 3-(4-Fluoro-2-phenethoxyphenyl)pyridine (12)

The title compound was synthesized from 1-bromo-4-fluoro-2-phenethoxybenzene 12a (118 mg, 0.40 mmol) according to method D using 3-pyridylboronic acid. The crude product was purified by flash chromatography on silica gel using a mixture of hexane / ethyl acetate (5:1) as eluent. Yield: 86 mg (73%); brown oil; $R_f = 0.66$ (n-Hex/EtOAc 1:2); Anal. C19H16FNO (C, H, N, O). IR (ATR): $\tilde{\nu}$ (cm$^{-1}$) = 3029 (m), 2935 (m), 1599 (s), 1279 (s), 1160 (vs), 699 (vs); $^1$H-NMR (CDCl$_3$, 500 MHz): $\delta_H = 8.73$ (d, $J = 1.9$ Hz, 2H), 7.69 (d, $J = 1.4$ Hz, 1H), 7.21–7.34 (m, 5H), 7.15 (d, $J = 7.2$ Hz, 2H), 6.70–6.76 (m, 2H), 4.19 (t, $J = 6.5$ Hz), 3.03 (t, $J = 6.5$ Hz); $^{13}$C-NMR (CDCl$_3$, 125 MHz); $\delta_C = 162.9$ (d, $J_{CF} = 244.7$ Hz), 156.7, 149.5, 147.8,
138.2, 136.6, 132.8, 131.4 (d, \(J_{CF} = 10.1\) Hz), 136.9, 123.0, 117.5, 107.2 (d, \(J_{CF} = 21.1\) Hz), 100.7 (d, \(J_{CF} = 26.6\) Hz), 69.1, 34.6; MS (ESI): \(m/z = 293.87\) [M+H]^+.

4.1.18. 4-(4-Fluoro-2-phenethoxyphenyl)pyridine (13)

The title compound was synthesized from 1-bromo-4-fluoro-2-phenethoxybenzene 12a (128 mg, 0.43 mmol) according to method D using 4-pyridylboronic acid. The crude product was purified by flash chromatography on silica gel using a mixture of hexane / ethyl acetate (5:1) as eluent. Yield: 89 mg (71 %); fawn oil; \(R_f = 0.54\) (n-Hex/EtOAc 1:2); Anal. C19H16FNO (C, H, N, O). IR (ATR): \(\tilde{\nu}\) (cm\(^{-1}\)) = 3029 (m), 2936 (m), 1599 (vs), 1407 (s), 1280 (vs), 1161 (vs), 832 (vs), 699 (vs); \(^1\)H-NMR (CDCl\(_3\), 500 MHz): \(\delta_H = 8.54\) (dd, \(J = 4.7, 1.6\) Hz, 2H), 7.21–7.31 (m, 6H), 7.14–7.18 (m, 2H), 6.67–6.75 (m, 2H), 4.20 (t, \(J = 6.5\) Hz, 2H), 3.03 (t, \(J = 6.5\) Hz, 2H); \(^13\)C-NMR (CDCl\(_3\), 125 MHz) \(\delta_C = 35.4, 69.4, 100.4\) (d, \(J_{CF} = 25.7\) Hz), 107.7 (d, \(J_{CF} = 22.0\) Hz), 123.6 (d, \(J_{CF} = 3.7\) Hz), 124.4, 126.7, 128.5, 129.0, 131.3 (d, \(J_{CF} = 10.1\) Hz), 137.8, 146.0, 148.8, 157.0 (d, \(J_{CF} = 9.2\) Hz), 163.9 (d, \(J_{CF} = 248.4\) Hz); MS (ESI): \(m/z = 293.91\) [M+H]^+.

4.1.19. 3-(4-Methoxy-2-phenethoxyphenyl)pyridine (14)

The title compound was synthesized from 1-bromo-4-methoxy-2-phenethoxybenzene 14a (100 mg, 0.33 mmol) according to method C using 3-pyridylboronic acid. The crude product was purified by flash chromatography on silica gel using a mixture of petroleum ether / ethyl acetate (2:1) as eluent. Yield: 78 mg (77 %); lemon yellow oil; \(R_f = 0.55\) (n-Hex/EtOAc 1:2); Anal. C20H19NO2 (C, H, N, O). IR (ATR): \(\tilde{\nu}\) (cm\(^{-1}\)) = 3028 (m), 2934 (m), 2836 (m), 1883 (w), 1608 (s), 1165 (vs), 699 (vs); \(^1\)H-NMR (CDCl\(_3\), 500 MHz): \(\delta_H = 8.72\) (d, \(J = 1.9\) Hz, 1H), 8.55 (dd, \(J = 1.6, 4.7\) Hz, 1H), 7.71–7.75 (m, 1H), 7.24–7.28 (m, 5H), 7.19–7.22 (m, 2H), 6.62 (dd, \(J = 2.2, 8.4\) Hz, 1H), 6.58 (d, \(J = 2.2\) Hz, 1H), 4.23 (t, \(J = 6.6\) Hz, 2H), 3.87 (s, 3H), 3.07 (t, \(J = 6.6\) Hz, 2H); \(^13\)C-NMR (CDCl\(_3\), 125 MHz): \(\delta_C = 161.0, 156.9, 149.5, 146.7, 138.1, 137.4, 134.3, 131.1, 129.0, 128.4, 126.5, 122.8, 119.8, 105.3, 99.8, 69.2, 55.4, 35.6; MS (ESI): \(m/z = 305.85\) [M+H]^+. 22
4.1.20. 4-(4-Methoxy-2-phenethoxyphenyl)pyridine (15)

The title compound was synthesized from 1-bromo-4-methoxy-2-phenethoxybenzene 14a (106 mg, 0.35 mmol) according to method C using 4-pyridylboronic acid. The crude product was purified by flash chromatography on silica gel using a mixture of petroleum ether / ethyl acetate (1:1) as eluent. Yield: 76 mg (71%); lemon yellow solid: mp 63–64 °C; \( R_f = 0.40 \) (n-Hex/EtOAc 1:2); Anal. C20H19NO2 (C, H, N, O). IR (ATR): \( \overline{\nu} \) (cm\(^{-1}\)) = 3028 (m), 2955 (m), 2835 (m), 1941 (w), 1610 (s), 1207 (vs), 783 (vs); \(^1\)H-NMR (CDCl\(_3\), 500 MHz): \( \delta_H = 8.47 \text{(dd, } J = 1.6, 4.7 \text{ Hz, } 2\text{H}), 7.17–7.28 (\text{m, } 6\text{H}), 7.13–7.17 (\text{m, } 2\text{H}), 6.53 \text{(dd, } J = 2.5, 8.5 \text{ Hz, } 1\text{H}), 6.49 \text{(d, } J = 2.5 \text{ Hz, } 1\text{H}), 4.17 \text{(t, } J = 6.5 \text{ Hz, } 2\text{H}), 3.79 \text{(s, } 3\text{H}), 3.00 \text{(t, } J = 6.5 \text{ Hz, } 2\text{H}); ^{13}\text{C-NMR (CDCl}_3\text{, 125 MHz): } \delta_C = 161.5, 157.0, 148.8, 146.5, 138.1, 131.1, 129.0, 128.5, 126.6, 124.2, 120.4, 105.4, 99.8, 69.1, 55.4, 35.6; MS (ESI): m/z = 305.86 [M+H]^+.

4.1.21. 3-(2-Phenethoxy-4-(trifluoromethyl)phenyl)pyridine (16)

The title compound was synthesised from 1-bromo-2-phenethoxy-4-(trifluoromethyl)benzene 16a (106 mg, 0.31 mmol) according to method C using 3-pyridylboronic acid. The crude product was purified by flash chromatography on silica gel using a mixture of hexane / ethyl acetate (2:1) as eluent. Yield: 75 mg (70%); light yellow solid: mp = 43–44 °C; \( R_f = 0.68 \) (n-Hex/EtOAc 1:2); Anal. C20H16F3NO (C, H, N, O). IR (ATR): \( \overline{\nu} \) (cm\(^{-1}\)) = 3031 (m), 2949 (m), 1614 (m), 1406 (s), 1328 (vs), 1105 (vs) 698 (vs); \(^1\)H-NMR (CDCl\(_3\), 500 MHz): \( \delta_H = 8.68 \text{(dd, } J = 0.8, 2.4 \text{ Hz, } 1\text{H}), 8.60 \text{(dd, } J = 1.6, 4.7 \text{ Hz, } 1\text{H}), 7.66–7.69 (\text{m, } 1\text{H}), 7.37–7.40 (\text{m, } 1\text{H}), 7.20–7.31 (\text{m, } 5\text{H}), 7.15–7.17 (\text{m, } 1\text{H}), 7.12–7.15 (\text{m, } 2\text{H}), 4.24 \text{(t, } J = 6.6 \text{ Hz, } 2\text{H}), 3.03 \text{(t, } J = 6.6 \text{ Hz, } 2\text{H}); ^{13}\text{C-NMR (CDCl}_3\text{, 125 MHz): } \delta_C = 156.0, 149.8, 148.4, 137.8, 137.2, 133.0, 131.5 (q, } J_{CF} = 32.5 \text{ Hz), 130.9, 130.6, 129.0, 128.5, 126.6, 122.9, 123.8 (q, } J_{CF} = 272.2 \text{ Hz), 117.8 (q, } J_{CF} = 3.7 \text{ Hz), 108.8 (q, } J_{CF} = 3.7 \text{ Hz), 69.5, 35.5; MS (ESI): m/z = 343.81 [M+H]^+.

4.1.22. 4-(2-Phenethoxy-4-(trifluoromethyl)phenyl)pyridine (17)
The title compound was synthesized from 1-bromo-2-phenethoxy-4-(trifluoromethyl)benzene \(16a\) (100 mg, 0.29) according to method C using 4-pyridylboronic acid. The crude product was purified by flash chromatography on silica gel using a mixture of petroleum ether / ethyl acetate (4:1) as eluent. Yield: 73 mg (73 %); light fawn solid: mp = 58–59 °C; \(R_f = 0.55\) (n-Hex/EtOAc 1:2); Anal. C20H16F3NO (C, H, N, O). IR (ATR): \(\mathbf{\nu} (\text{cm}^{-1}) = 3033\) (m), 2946 (m), 1591 (s), 1329 (vs), 1109 (vs), 817 (vs); \(^1\)H-NMR (CDCl\(_3\), 500 MHz): \(\delta_H = 8.57\) (dd, \(J = 1.6, 4.4\) Hz, 2H), 7.37 (dd, \(J = 7.9, 0.6\) Hz, 1H), 7.24–7.29 (m, 5H), 7.20–7.24 (m, 1H), 7.12–7.17 (m, 3H), 4.25 (t, \(J = 6.3\) Hz, 2H), 3.02 (t, \(J = 6.3\) Hz, 2H); \(^13\)C-NMR (CDCl\(_3\), 125 MHz): \(\delta_C = 155.9, 149.2, 145.2, 137.8, 132.1\) (q, \(J_{CF} = 33.0\) Hz), 131.1, 130.8, 129.0, 128.5, 126.7, 124.3, 123.7 (q, \(J_{CF} = 272.2\) Hz), 117.8 (q, \(J_{CF} = 3.7\) Hz), 108.9 (q, \(J_{CF} = 3.7\) Hz), 69.5, 35.5; MS (ESI): \(m/z = 343.82\) [M+H].

4.1.23. 3-Phenethoxy-4-(pyridin-3-yl)benzonitrile (18)

The title compound was synthesized from 4-bromo-3-phenethoxybenzonitrile \(18a\) (105 mg, 0.35 mmol) according to method D using 3-pyridylboronic acid. The crude product was purified by flash chromatography on silica gel using a mixture of petroleum ether / ethyl acetate (1.5:1) as eluent. Yield: 82 mg, (78 %); brown oil; \(R_f = 0.56\) (n-Hex/EtOAc 1:2); Anal. C20H16N2O (C, H, N, O). IR (ATR): \(\mathbf{\nu} (\text{cm}^{-1}) = 3029\) (m), 2936 (m), 2228 (s), 1506 (s), 1397 (s), 1277 (vs), 1021 (s), 700 (vs); \(^1\)H-NMR (CDCl\(_3\), 500 MHz): \(\delta_H = 8.7\) (br, s, 1H) 8.6 (br, s, 1H) 7.7 (d, \(J = 7.6\) Hz, 1H) 7.3–7.4 (m, 3H) 7.2–7.3 (m, 4H) 7.1–7.2 (m, 2H) 4.3 (t, \(J = 6.5\) Hz, 2H) 3.1 (t, \(J = 6.5\) Hz, 2H); \(^13\)C-NMR (CDCl\(_3\), 125 MHz): \(\delta_C = 156.0, 149.2, 148.2, 137.5, 134.4, 132.8, 131.8, 131.2, 128.9, 128.5, 126.7, 125.0, 123.1, 118.4, 115.1, 113.0, 69.6, 35.4; MS (ESI): \(m/z = 300.96\) [M+H].

4.1.24. 3-Phenethoxy-4-(pyridin-4-yl)benzonitrile (19)

The title compound was synthesized from 4-bromo-3-phenethoxybenzonitrile \(18a\) (110 mg, 0.36 mmol) according to method D using 4-pyridylboronic acid. The crude product was purified by flash chromatography on silica gel using a mixture of petroleum ether / ethyl
acetate (1.5:1) as eluent. Yield: 92 mg, (85 %); white solid: mp = 112–113 °C; \( R_f = 0.44 \) (n-Hex/EtOAc 1:2); Anal. C20H16N2O (C, H, N, O). IR (ATR): \( \bar{\nu} \) (cm\(^{-1}\)) = 3031 (m), 2928 (m), 2228 (s), 1595 (s), 1277 (s), 1024 (s), 808 (vs), 752 (vs), 705 (vs); \(^1\)H-NMR (CDCl\(_3\), 500 MHz): \( \delta_H = 8.6 \) (d, \( J = 6.0 \) Hz, 2H), 7.4 (d, \( J = 7.9 \) Hz, 1H), 7.3 (dd, \( J = 1.6, 7.6 \) Hz, 1H), 7.2–7.3 (m, 5H), 7.2 (d, \( J = 1.6 \) Hz, 1H) 7.1–7.2 (m, 2H), 4.3 (t, \( J = 6.5 \) Hz, 2H), 3.1 (t, \( J = 6.5 \) Hz, 2H); \(^13\)C-NMR (CDCl\(_3\), 125 MHz): \( \delta_C = 155.9, 149.4, 144.5, 137.6, 132.6, 131.1, 128.9, 128.5, 126.8, 124.9, 124.1, 118.4, 115.2, 113.4, 69.6, 35.4 \); MS (ESI): \( m/z = 300.96 \) [M+H]\(^+\).

4.1.25. \( \text{N,N-Dimethyl-3-phenethoxy-4-(pyridin-3-yl)benzamide (20)} \)

The title compound was synthesised from 4-bromo-N,N-dimethyl-3-phenethoxybenzamide 20a (100 mg, 0.29 mmol) according to method C using 3-pyridylboronic acid. The crude product was purified by flash chromatography on silica gel using ethyl acetate as eluent. Yield: 95 mg (95 %); fawn solid: mp = 161–162 °C; \( R_f = 0.05 \) (n-Hex/EtOAc 1:2); Anal. C22H22N2O2 (C, H, N, O). IR (ATR): \( \bar{\nu} \) (cm\(^{-1}\)) = 3451 (br, m), 2927 (m), 1711 (m), 1609 (s), 1406 (s), 1329 (vs), 703 (s); \(^1\)H-NMR ((CD\(_3\))\(_2\)CO, 500 MHz): \( \delta_H = 8.69 \) (dd, \( J = 1.0, 2.2 \) Hz, 1H), 8.54 (dd, \( J = 1.6, 4.7 \) Hz, 1H), 7.78 (ddd, \( J = 1.9, 3.5, 7.9 \) Hz, 1H), 7.40 (d, \( J = 7.9 \) Hz, 1H), 7.36 (ddd, \( J = 0.8, 4.9, 7.9 \) Hz, 1H), 7.16 (d, \( J = 1.6 \) Hz, 1H), 7.09 (dd, \( J = 1.4, 7.7 \) Hz, 1H), 4.33 (t, \( J = 6.5 \) Hz, 2H), 3.02–3.07 (m, 8H); \(^13\)C-NMR ((CD\(_3\))\(_2\)CO, 125 MHz): \( \delta_C = 171.1, 156.8, 150.9, 149.1, 139.6, 138.7, 137.7, 134.4, 131.1, 130.1, 129.2, 129.0, 127.2, 123.8, 120.6, 112.4, 70.2, 60.6, 36.3 \); MS (ESI): \( m/z = 346.81 \) [M+H]\(^+\).

4.1.26. \( \text{N,N-Dimethyl-3-phenethoxy-4-(pyridin-4-yl)benzamide (21)} \)

The title compound was synthesised from 4-bromo-N,N-dimethyl-3-phenethoxybenzamide 20a (96 mg, 0.28 mmol) according to method C using 4-pyridylboronic acid. The crude product was purified by flash chromatography on silica gel using ethyl acetate as eluent. Yield: 76 mg (78 %); white solid: mp = 262–263 °C; \( R_f = 0.05 \) (n-Hex/EtOAc 1:2); Anal. C22H22N2O2 (C, H, N, O). IR (ATR): \( \bar{\nu} \) (cm\(^{-1}\)) = 3438 (br, m), 2933 (m), 1609 (s), 1414 (s), 1315 (vs), 700 (s); \(^1\)H-NMR (DMSO-\(d_6\), 500 MHz): \( \delta_H = 8.53 \) (dd, \( J = 1.6, 4.5 \) Hz, 2H),
7.41 (d, $J = 7.6$ Hz, 1H), 7.35 (dd, $J = 1.6$, 4.5 Hz, 2H), 7.25–7.30 (m, 2H), 7.19–7.24 (m, 3H), 7.15 (d, $J = 1.6$ Hz, 1H), 7.05 (dd, $J = 1.6$, 7.6 Hz, 1H), 4.30 (t, $J = 6.5$ Hz, 2H), 2.85–3.05 (m, 8H); $^{13}$C-NMR (DMSO-$d_6$, 125 MHz): $\delta_C = 169.4$, 155.3, 149.3, 144.8, 138.5, 138.4, 130.2, 129.0, 128.2, 127.4, 126.3, 124.0, 119.4, 111.3, 68.9, 34.8; MS (ESI): $m/z = 346.80$ [M+H]$^+$.  

4.1.27. 3-Phenethoxy-2,3'-bipyridine (22)  
The title compound was synthesised from 2-bromo-3-phenethoxy pyridine 22a (95 mg, 0.342 mmol) according to method C using 3-pyridylboronic acid. The crude product was purified by flash chromatography on silica gel using ethyl acetate as eluent. Yield: 92 mg, (97.5 %); light yellow solid: mp = 126–127 °C; $R_f = 0.14$ (n-Hex/EtOAc 1:2); Anal. C18H16N2O (C, H, N, O). IR (ATR): $\bar{\nu}$ (cm$^{-1}$) = 3510 (br, m), 2990 (m), 1579 (m), 1452 (s), 1321 (vs), 696 (s); $^1$H-NMR (CDCl$_3$, 500 MHz): $\delta_H = 9.2$ (dd, $J = 0.6$, 2.2 Hz, 1H), 8.6 (dd, $J = 1.6$, 5.0 Hz, 1H), 8.3 (dd, $J = 1.4$, 4.6 Hz, 1H), 8.2 (dt, $J = 2.0$, 8.0 Hz, 1H), 7.4 (ddd, $J = 0.8$, 4.8, 8.0 Hz, 1H), 7.3–7.3 (m, 3H), 7.2–7.3 (m, 4H), 4.3 (t, $J = 6.6$ Hz, 2H), 3.1 (t, $J = 6.6$ Hz, 2H); $^{13}$C-NMR (CDCl$_3$, 125 MHz): $\delta_C = 153.2$, 149.8, 148.2, 144.7, 141.8, 137.6, 137.4, 133.7, 129.0, 128.6, 126.8, 123.8, 123.0, 119.5, 69.4, 35.6; MS (ESI): $m/z = 276.95$ [M+H]$^+$.  

4.1.28. 3-Phenethoxy-2,4'-bipyridine (23)  
The title compound was synthesised from 2-bromo-3-phenethoxy pyridine 22a (50 mg, 0.18 mmol) according to method D using 4-pyridylboronic acid. The crude product was purified by flash chromatography on silica gel using ethyl acetate as eluent. Yield: 35 mg, (70.4 %); light brown solid: mp = 39–40 °C; $R_f = 0.16$ (n-Hex/EtOAc 1:2); Anal. C18H16N2O (C, H, N, O). IR (ATR): $\bar{\nu}$ (cm$^{-1}$) = 3454 (br, m), 1642 (m), 1437 (s), 1432 (vs), 1272 (s), 814 (s), 744 (s), 699 (s); $^1$H-NMR (CDCl$_3$, 500 MHz): $\delta_H = 8.6$ (d, $J = 4.7$ Hz, 2H), 8.3 (dd, $J = 1.6$, 4.4 Hz, 1H), 7.7 (d, $J = 5.4$ Hz, 2H), 7.2–7.3 (m, 7H), 4.3 (t, $J = 6.6$ Hz, 2H), 3.1 (t, $J = 6.6$ Hz, 2H); $^{13}$C-NMR (CDCl$_3$, 125 MHz): $\delta_C = 153.7$, 149.6, 145.3, 145.1, 141.9, 137.9, 129.2, 128.8, 127.0, 124.6, 124.0, 119.9, 69.5, 35.8; MS (ESI): $m/z = 276.97$ [M+H]$^+$.  

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4.1.29. 3-(4-Nitro-2-phenethoxybenzyl)pyridine (24)

The title compound was synthesized from 4,4,5,5-tetramethyl-2-(4-nitro-2-phenethoxyphenyl)-1,3,2-dioxaborolane 24a (97 mg, 0.26 mmol) according to method D using 3-pyridylboronic acid. The crude product was purified by flash chromatography on silica gel using a mixture of hexane / ethyl acetate (2:1) as eluent. Yield: 55 mg (63 %); light brown solid: mp = 82–83 °C; Rf = 0.48 (n-Hex/EtOAc 1:2); Anal. C20H18N2O3 (C, H, N, O). IR (ATR): $\tilde{\nu}$ (cm$^{-1}$) = 3061 (m), 2928 (m), 2879 (m), 1585 (m), 1511 (vs), 1337 (vs), 1248 (vs), 1026 (s), 744 (vs), 704 (vs); $^1$H-NMR (CDCl$_3$, 500 MHz): $\delta$H = 8.48 (br, s, 2H), 7.76 (dd, J = 8.2, 2.2 Hz, 1H), 7.30 (d, J = 2.2 Hz, 1H), 7.38 (d, J = 7.9 Hz, 1H), 7.30–7.35 (m, 2H), 7.24–7.28 (m, 3H), 7.22 (d, J = 8.2 Hz, 1H), 7.18 (dd, J = 7.4, 4.9 Hz, 1H), 4.30 (t, J = 6.6 Hz, 2H), 3.95 (s, 2H), 3.13 (t, J = 6.6 Hz, 2H); $^{13}$C-NMR (CDCl$_3$, 125 MHz) $\delta$C = 156.6, 149.8, 147.8, 147.4, 137.6, 136.5, 136.0, 134.9, 130.3, 128.8, 128.6, 126.7, 123.5, 115.9, 106.2, 69.3, 35.5, 33.4; MS (ESI): m/z = 335.03 [M+H]$^+$. 

4.1.30. 4-(4-Nitro-2-phenethoxybenzyl)pyridine (25)

The title compound was synthesized from 4,4,5,5-tetramethyl-2-(4-nitro-2-phenethoxyphenyl)-1,3,2-dioxaborolane 24a (80 mg, 0.22 mmol) according to method D using 4-pyridylboronic acid. The crude product was purified by flash chromatography on silica gel using a mixture of hexane / ethyl acetate (2:1) as eluent. Yield: 51 mg (69 %); brown oil; Anal. C20H18N2O3 (C, H, N, O). Rf = 0.35 (n-Hex/EtOAc 1:2); IR (ATR): $\tilde{\nu}$ (cm$^{-1}$) = 3028 (m), 2923 (m), 2851 (m), 1598 (m), 1517 (vs), 1341 (vs), 1247 (vs), 801 (s), 738 (vs), 700 (vs); $^1$H-NMR (CDCl$_3$, 500 MHz): $\delta$H = 8.45 (d, J = 6.0 Hz, 2H), 7.82 (dd, J = 8.2, 2.2 Hz, 1H), 7.74 (d, J = 2.2 Hz, 1H), 7.25–7.33 (m, 5H), 7.21 (dd, J = 7.7, 1.7 Hz, 2H), 7.10 (d, J = 6.3 Hz, 2H), 4.32 (t, J = 6.5 Hz, 2H), 4.02 (s, 2H), 3.10 (t, J = 6.5 Hz, 2H); $^{13}$C-NMR (CDCl$_3$, 125 MHz) $\delta$C = 156.7, 152.5, 148.2, 147.0, 140.7, 137.5, 134.1, 130.8, 128.7, 126.9, 124.7, 116.1, 106.4, 69.2, 35.9, 35.4; MS (ESI): m/z = 334.99 [M+H]$^+$. 

4.2. Biology
4.2.1. Inhibition of \( h\text{CYP11B1}, h\text{CYP11B2} \) and \( r\text{CYP11B1} \)

\( V79\text{MZ}h\text{CYP11B1}, V79\text{MZ}h\text{CYP11B2} \) or \( V79\text{MZ}r\text{CYP11B1} \) cells were pre-incubated with inhibitor for 1 h at 37 °C. The reaction was started by addition of 100 nM (\( h\text{CYP11B} \)) or 500 nM (\( r\text{CYP11B1} \)) \([^3]H\)-11-deoxycorticosterone as substrate. After incubation for 25 min (\( h\text{CYP11B1} \)), 45 min (\( h\text{CYP11B2} \)) or 7 h (\( r\text{CYP11B1} \)), the enzyme reactions were stopped by extracting the supernatant with ethyl acetate. Samples were centrifuged, and the ethyl acetate was separated. [46,50] The steroids were separated by HPLC and analyzed with radio flow detection.

4.2.2. Inhibition of \( h\text{CYP19} \)

The inhibition of \( h\text{CYP19} \) was determined in vitro using human placental microsomes with \([1\beta-[^3]H]\text{androstenedione} \) as substrate. [48]

4.2.2. Inhibition of \( h\text{CYP17} \)

Human CYP17 was expressed in \( E.\text{coli} \) (coexpressing human CYP17 and NADPH-P450 reductase) and the assay was performed as previously described. [49]

4.3. Modelling studies

4.3.1. Establishment of \( h\text{CYP11B1} \) homology model

The primary sequence of CYP11B1 \( \text{Homo sapiens} \) (UniProt code: P15538) was obtained from the Universal Protein Resource (UniProt, http://www.uniprot.org/), which was subsequently subject to similarity search in the UniProtKB database using the Basic Local Alignment Search Tool (BLAST) sever in UniProt. Among the hits, \( h\text{CYP11B2} \) (UniProt code: P19099) with a crystal in complex with deoxycorticosterone (PDB ID: 4DVQ, chain A) that showed 93.2 % identity and an E-value of 0.0 was selected as the template. The target and template proteins subsequently underwent an tree-based initial sequence alignment and then structural realignment taking into consideration the coordinates of alpha carbons in the \( h\text{CYP11B2} \) crystal using MOE. After setting the force field to Amber99 with Solvation R Field, a library of ten initial models was generated employing the Generalized Born/Volume Integral (GB/VI)
scoring function, from which the best one was selected according to electrostatic solvation energy and root mean square deviation of each intermediate model to the average position of all models.

4.3.2. Molecular dynamic simulation

The force field of Amber99 was firstly applied to the preliminary model, and the model was partially charged and energy-minimized to RMS Gradient of 0.1 before the initiation of molecular dynamic simulation using MOE. Conformations were generated in the statistical ensemble with thermodynamic variables of volume, temperature and number of particles being held as constants. The method of the Nosé-Poincaré-Anderson equations of motion was employed to create ensemble trajectory. The system was heated from 0K to 300K in 60 picoseconds and, after a run time of 1 nanosecond, it was cooled down to 0K in 60 picoseconds.

4.3.3. Molecular docking

The homology model was prepared by adding hydrogens and partial charges using the Protonate3D module in MOE. Compound 8 as the ligand was built and energy-minimized in the MMFF94s force field with MOE. After importing both the protein model and the ligand into GOLD, the heme iron was appointed as the origin of active-site with a radius 19 Å, although the function of automatic active-site detection was also switched on. A distance constraint of 1.5−4.5 Å between the heme iron and the coordinating N was set. Ligand was docked in 50 independent genetic algorithm iterations for each run with default parameters. The resulting binding poses were evaluated using the goldscore function with p450_pdb parameters and subsequently ranked according to fitness. The diverse binding modes were further investigated and illustrated with the LigX module in MOE.

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We thank Jeannine Jung for performing the in vitro tests and are grateful to Prof. J. J. Rob Hermans, University of Maastricht, The Netherlands, for providing the V79MZhCYP11B1 cells.

**Supplementary Data** Experimental details and spectroscopic characterization of all intermediates as well as the $^1$H- and $^{13}$C-NMR spectra of the most potent and selective compounds 4–9, 18, 19 and 25.

**References**


30


[37] Leze, M. P.; Le Borgne, M.; Pinson, P.; Palusczak, A.; Duflos, M.; Le Baut, G.; Hartmann, R. W. Synthesis and biological evaluation of 5-[(aryl)(1H-imidazol-1-yl)methyl]-


Chart 1. The structures of typical hCYP11B1 inhibitors (Metyrapone, LCl699, compounds I and II) as well as lead compounds III, IV and designed compounds 1-25.
Scheme 1: Syntheses of compounds 4–23. Reagents and conditions. (i) Method A: BBr₃, DCM, -20 to RT, overnight. (ii) for 22b: HBr, 140°C, overnight. (iii) Method B: corresponding brimide, K₂CO₃, KI, ethanol, reflux, overnight. (iv) for 10a: (2-bromoethyl)benzene, tetrabutylammonium iodide, DMF, Cs₂CO₃, 140°C, 3h. (v) for 4–9, 14–17 and 20–22: Method C: Pd(PPh₃)₄, corresponding pyridyl boronic acid, Na₂CO₃, ethanol, toluene, H₂O, reflux, 3-5h. (vi) for 10–13, 18, 19 and 23: Method D: PdCl₂(dppf), corresponding pyridyl boronic acid, Na₂CO₃, DME, H₂O, reflux, overnight.

Scheme 2: Syntheses of compounds 1–3. Reagents and conditions. (i) hydrazine hydrate, Pd/C, EtOH, reflux, 1h. (ii) acetyl chloride or 4-fluorobenzoyl chloride, triethylamine, N,N-dimethylamionpyridine, dichloromethane, RT, overnight.

Scheme 3: Syntheses of compounds 24 and 25. Reagents and conditions. (i) bis(pinacolato)diboron, PdCl₂(PPh₃)₂, KOAc, dioxane, reflux, 4h. (ii) Method D: PdCl₂(dppf), corresponding pyridyl boronic acid, Na₂CO₃, DME, H₂O, reflux, 4h.
**Table 1.** Inhibition of hCYP11B1, hCYP11B2, hCYP19 and rCYP11B1 by compounds 1-25 as well as references I-IV and metyrapone.

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<th>SF_d</th>
<th>IC_{50} (nM)^a</th>
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a Mean value of at least three experiments, standard deviation less than 25 %.
b Hamster fibroblasts expressing hCYP11B1, hCYP11B2 or rCYP11B1, respectively; substrate deoxycorticosterone, 100 nM for hCYP11B1 and hCYP11B2 and 500 nM for rCYP11B1.
c Human placental microsomes, substrate androstenedione, 500 nM.
d SF = IC_{50 \text{hCYP11B2}} / IC_{50 \text{hCYP11B1}}.
e Met: Metyrapone.
Figure 1. (A) Superposition of the hCYP11B1 homology model (green) onto the hCYP11B2 crystal structure (magenta, PDB ID: 4DVQ-A). (B) Comparison of the active sites. (C) Configurational changes of F130 and L131 strongly influenced the hydrophobic pocket delimitated by B’-C loop, B-B’ loop and β2-sheet.
Figure 2. Docking of compound 8 (magenta) in the hCYP1B1 homology model. Interactions between the inhibitor and the enzyme are depicted in gold dashed lines.