Overcoming Undesirable CYP1A2 Inhibition of Pyridylnaphthalene-Type Aldosterone Synthase Inhibitors: Influence of Heteroaryl Derivatization on Potency and Selectivity

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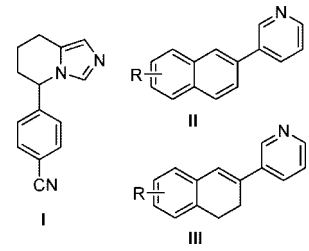
Recently, we reported on the development of potent and selective inhibitors of aldosterone synthase (CYP11B2) for the treatment of congestive heart failure and myocardial fibrosis. A major drawback of these nonsteroidal compounds was a strong inhibition of the hepatic drug-metabolizing enzyme CYP1A2. In the present study, we examined the influence of substituents in the heterocycle of lead structures with a naphthalene molecular scaffold to overcome this unwanted side effect. With respect to CYP11B2 inhibition, some substituents induced a dramatic increase in inhibitory potency. The methoxyalkyl derivatives **22** and **26** are the most potent CYP11B2 inhibitors up to now (IC_{50} = 0.2 nM). Most compounds also clearly discriminated between CYP11B2 and CYP11B1, and the CYP1A2 potency significantly decreased in some cases (e.g., isoquinoline derivative **30** displayed only 6% CYP1A2 inhibition at 2 μM concentration). Furthermore, isoquinoline derivative **28** proved to be capable of passing the gastrointestinal tract and reached the general circulation after peroral administration to male Wistar rats.

Introduction

The most important circulating mineralocorticoid aldosterone is secreted by the zona glomerulosa of the adrenal gland and is to a minor extent also synthesized in the cardiovascular system.[1] The hormone plays a key role in the electrolyte and fluid homeostasis and thus for the regulation of blood pressure. Its biosynthesis is accomplished by the mitochondrial cytochrome P450 enzyme aldosterone synthase (CYP11B2[a]) and proceeds via catalytic oxidation of the substrate 11-deoxycorticosterone to corticosterone and subsequently to aldosterone.[2] The adrenal aldosterone synthesis is regulated by several physiological parameters such as the renin−angiotensin−aldosterone system (RAAS) and the plasma potassium concentration. Chronically elevated plasma aldosterone levels increase the blood pressure and are closely associated with certain forms of myocardial fibrosis and congestive heart failure.[3] An insufficient renal flow chronically activates the RAAS, and aldosterone is excessively released. The therapeutic benefit of reducing aldosterone effects by use of the mineralocorticoid receptor (MR) antagonists spironolactone and eplerenone has been reported in two recent clinical studies (RALES and EPHESUS).[4,5] The studies showed that treatment with these antagonists reduces mortality in patients with chronic congestive heart failure and in patients after myocardial infarction, respectively. Spironolactone, however, showed severe side effects, presumably due to its steroidal structure.[4,6] Although the development of nonsteroidal aldosterone receptor antagonists has been reported recently,[7] several issues associated with the unaffected and pathophysiologically elevated plasma aldosterone levels remain unsolved by this therapeutic strategy such as the up-regulation of the mineralocorticoid receptor expression[8] and nongenomic aldosterone effects.[9] A novel approach for the treatment of diseases affected by elevated aldosterone levels is the blockade of aldosterone biosynthesis by inhibition of CYP11B2.[10,11] Aldosterone synthase has previously been proposed as a potential pharmacological target,[12] and preliminary work focused on the development of steroidal inhibitors, i.e., progesterone[13] and deoxycorticosterone[14] derivatives with unsaturated C_{18}-substituents. These compounds were found to be mechanism-based inhibitors binding covalently to the active site of bovine CYP11B, however, data on inhibitory action toward human enzyme are essentially absent in these studies. The strategy of inhibiting the aldosterone formation has two main advantages compared to MR antagonism. First, there is no nonsteroidal inhibitor of a steroidogenic CYP enzyme known to have affinity to a steroid receptor. For this reason, fewer side effects on the endocrine system should be expected. Furthermore, CYP11B2 inhibition can reduce the pathologically elevated aldosterone levels, whereas the latter remain unaffected by interfering one step later at the receptor level. By this approach, however, it is a challenge to reach selectivity versus other CYP enzymes. Taking into consideration that the key enzyme of glucocorticoid biosynthesis, 11β-hydroxylase (CYP11B1) and CYP11B2 have a sequence homology of more than 93%,[15] the selectivity issue becomes especially critical for the design of CYP11B2 inhibitors.

Chart 1. Nonsteroidal Inhibitors of CYP11B2



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[a] Abbreviations: CYP11B2, aldosterone synthase; CYP11B1, 11β-hydroxylase; CYP17, 17α-hydroxylase-17,20-lyase; CYP19, aromatase; CYP, cytochrome P450; RAAS, renin-angiotensin-aldosterone system; MR, mineralocorticoid receptor; AUC, area under the curve; MEP, molecular electrostatic potential.

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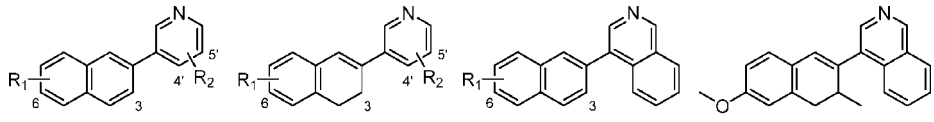
Table 1. Inhibition of Human Adrenal CYP11B2, CYP11B1, and Human CYP1A2 in Vitro

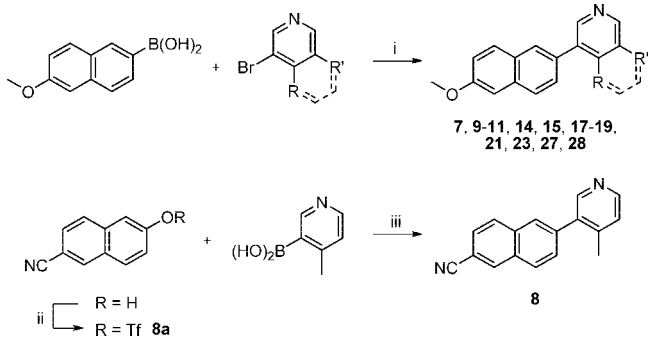


1-3, **7-12**, **14-27** **4-6**, **13** **28**, **29** **30**

			IC_{50} value[a] (nM)			
compd	R_1	R_2	V79 11B2[b] hCYP11B2	V79 11B1[c] hCYP11B1	selectivity factor[d]	% inhibition[e] CYP1A2[f]
1[g]	6-OMe	H	6.2	1577	254	98
2	6-OMe-3-Me	H	7.0	1047	150	93
3[g]	6-CN	H	2.9	691	239	97
4[g]	6-OMe	H	2.1	578	275	98
5	6-OMe-3-Me	H	3.3	248	79	73
6	6-CN	H	4.5	461	103	91
7	6-OMe	4′-Me	0.8	114	143	98
8	6-CN	4′-Me	0.6	52	87	86
9	6-OMe	4′-NH_2	13	1521	117	58
10	6-OMe	5′-OH	94	8925	95	93
11	6-OMe	5′-OMe	4.2	238	57	91
12	6-OMe-3-Me	5′-OMe	3.8	875	230	91
13	6-OMe-3-Me	5′-OMe	1.2	100	83	18
14	6-OMe	5′-OEt	5.1	373	73	85
15	6-OMe	5′-COOH	n.a.[h]	n.d.	n.d.	n.d.
16	6-OMe	5′-COOMe	0.8	15	19	n.d.
17	6-OMe	5′-$CONH_2$	94	41557	442	n.d.
18	6-OMe	5′-COMe	2.1	255	121	80
19	6-OMe	5′-CH_2COOH	1216	37796	31	n.d.
20	6-OMe	5′-CH_2COOMe	6.9	199	29	n.d.
21	6-OMe	5′-CH_2OH	9.1	614	68	93
22	6-OMe	5′-CH_2OMe	0.2	31	155	83
23	6-OMe	4′-CH_2OH	22	1760	80	92
24	6-OMe	4′-CH_2OMe	2.2	435	198	97
25	6-OMe	5′-CH(OH)Me	0.5	99	198	78
26	6-OMe	5′-CH(OMe)Me	0.2	10	50	n.d.
27	6-OMe	5′-Ph	4.8	151	32	n.d.
28	6-OMe		0.6	67	112	57
29	6-OMe-3-Me		3.1	843	272	45
30			0.5	64	128	6
fadrozole			1.0	10	10	8

[a] Mean value of four experiments, standard deviation usually less than 25%, n.d. = not determined. [b] Hamster fibroblasts expressing human CYP11B2; substrate deoxycorticosterone, 100 nM. [c] Hamster fibroblasts expressing human CYP11B1; substrate deoxycorticosterone, 100 nM. [d] IC_{50} CYP11B1/IC_{50} CYP11B2. [e] Mean value of two experiments, standard deviation less than 5%; n.d. = not determined. [f] Recombinantly expressed enzyme from baculovirus-infected insect microsomes (Supersomes); inhibitor concentration, 2.0 μM; furafylline, 55% inhibition. [g] These compounds were described previously.[22,23] [h] n.a. = no activity (7% inhibition at an inhibitor concentration of 500 nM).

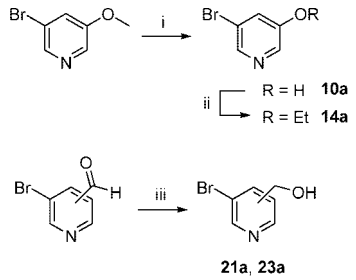
Scheme 1[a]



[a] Reagents and conditions: (i) $Pd(PPh_3)_4$, DMF, aq $NaHCO_3$, μw, 150 °C; (ii) Tf_2NPh, K_2CO_3, THF, μw, 120 °C; (iii) $Pd(dppf)Cl_2$, toluene/acetone, aq Na_2CO_3, μw, 150 °C.

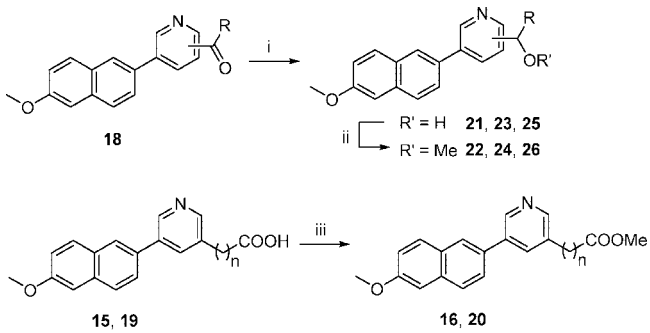
The aromatase (CYP19) inhibitor fadrozole (**I**, Chart 1), which is used in the therapy of breast cancer, was found to significantly reduce the corticoid formation.[16] This compound is a potent inhibitor of CYP11B2 displaying an IC_{50} value of 1 nM (Table 1). The R(+)-enantiomer of fadrozole (FAD 286) was recently shown to reduce mortality and to ameliorate angiotensin II-induced organ damage in transgenic rats overexpressing both the human renin and angiotensinogen genes.[17] These findings underline the potential therapeutic utility of aldosterone synthase inhibition and, up to now, several structurally modified fadrozole derivatives are investigated as CYP11B2 inhibitors.[18,19] Recently, the development of imidazolyl- and pyridylmethylenetetrahydronaphthalenes and -indanes as highly active and in some cases selective CYP11B2 inhibitors has been described by our group.[20,21] By keeping the pharmacophore and rigidization of the core structure, pyridine-substituted naphthalenes[22] **II** and dihydronaphthalenes[23] **III** were shown to be potent and selective CYP11B2 inhibitors (Chart 1). Combining the structural features of these substance classes to a hybrid core structure led to pyridine-substituted acenaphthenes as potent CYP11B2 inhibitors with remarkable selectivity.[24] Furthermore, most of the naphthalene and dihydronaphthalene type compounds exhibited a favorable selectivity profile versus selected hepatic CYP enzymes. However, they turned out to be potent inhibitors of the hepatic CYP1A2 enzyme (see examples **1**, **3**, and **4** in Table 1). CYP1A2 makes up about 10% of the overall cytochrome P450 content in the liver and metabolizes aromatic and heterocyclic amines as well as polycyclic aromatic hydrocarbons.[25] This experimental result turned these naphthalene type aldosterone synthase inhibitors into unsuitable drug candidates because adverse drug−drug interactions are mainly caused by inhibition of hepatic cytochrome P450 enzymes and

Scheme 2[a]



[a] Reagents and conditions: (i) conc HBr, reflux; (ii) EtBr, K_2CO_3, DMF, rt; (iii) $NaBH_4$, methanol, 0 °C.

Scheme 3[a]



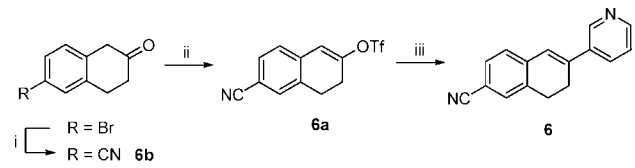
[a] Reagents and conditions: (i) $NaBH_4$, methanol, 0 °C; (ii) MeI, NaH, THF, rt; (iii) methanol, H_2SO_4, reflux.

have to be avoided in either case. In our preceding studies, the attention was focused on the optimization of the naphthalene skeleton; the substitution pattern of the heme complexing 3-pyridine moiety, however, was not investigated in detail. Herein, we describe the synthesis of a series of naphthalenes and dihydronaphthalenes with various substituents in the pyridine heterocycle to examine their influence on potency and selectivity (Table 1). The biological activity of the synthesized compounds was determined in vitro on human CYP11B2 for potency and human CYP11B1 and CYP1A2 for selectivity. In addition, selected compounds were tested for inhibitory activity at human CYP17 (17α-hydroxylase-C17,20-lyase), CYP19, and selected hepatic CYP enzymes (CYP2B6, CYP2C9, CYP2C19, CYP2D6, CYP3A4). The in vivo pharmacokinetic profile of two promising compounds was determined in a cassette dosing experiment using male Wistar rats.

Results

Chemistry. The key step for the synthesis of pyridine substituted naphthalenes was a Suzuki cross-coupling (Scheme 1).[26] A microwave enhanced method developed by van der Eycken et al. was chosen for this purpose.[27] By applying this method, various substituted bromopyridines were coupled with 6-methoxy-2-naphthaleneboronic acid to afford compounds **7**, **9**−**11**, **14**, **15**, **17**−**19**, **21**, **23**, **27**, and **28**. Compound **8** was obtained by coupling of 4-methyl-3-pyridineboronic acid with triflate **8a**, which was accessible by treating 6-cyano-2-naphthol with Tf_2NPh and K_2CO_3 in THF under microwave irradiation.[28] The bromopyridines could be derivatized prior to Suzuki coupling according to Scheme 2 to provide heterocycles bearing a hydroxy, ethoxy, or hydroxymethyl substituent (**10a**, **14a**, **21a**, and **23a**).[29] For compounds **21**−**26**, the substitution pattern was modified after the cross-coupling reaction as shown in Scheme 3 by sodium borohydride reduction and optional methylation. Esterification of the carboxylic acids **15** and **19** by refluxing in methanol under acid catalysis afforded the corresponding methyl esters **16** and **20**. The synthesis of 6-cyanodihydronaphthalene **6** was accomplished by the sequence shown in Scheme 4. Using 6-bromo-2-tetralone, Pd-catalyzed cyanation[30] led to intermediate **6b**, which was transformed into the alkenyltriflate **6a** by deprotonation with KHMDS and subsequent treatment with Tf_2NPh.[31] Compound **6a** underwent Suzuki coupling with 3-pyridineboronic acid to afford **6**. The naphthalenes **2**, **12**, and **29** and the dihydronaphthalenes **5**, **13**, and **30** were obtained as shown in Scheme 5. The sequence for the synthesis of intermediate **2e** was described previously and was only slightly modified by us (see Supporting Information).[32] Regioselective α-bromination was accomplished by treating **2e** with $CuBr_2$ in refluxing ethyl acetate/$CHCl_3$.[33] After a subsequent reduction/elimination step,[23] the intermediate alkenylbromide **2b** underwent Suzuki coupling[34] with the appropriate boronic acid to afford the dihydronaphthalenes **5**, **13**, and **30**. The corresponding naphthalenes **2**, **12**, and **29** were obtained by aromatization of **2b** with DDQ in refluxing toluene,[35] followed by Suzuki coupling.[27] The synthesis of compounds **1**, **3**, and **4** has been reported previously by our group.[22,23]

Scheme 4[a]



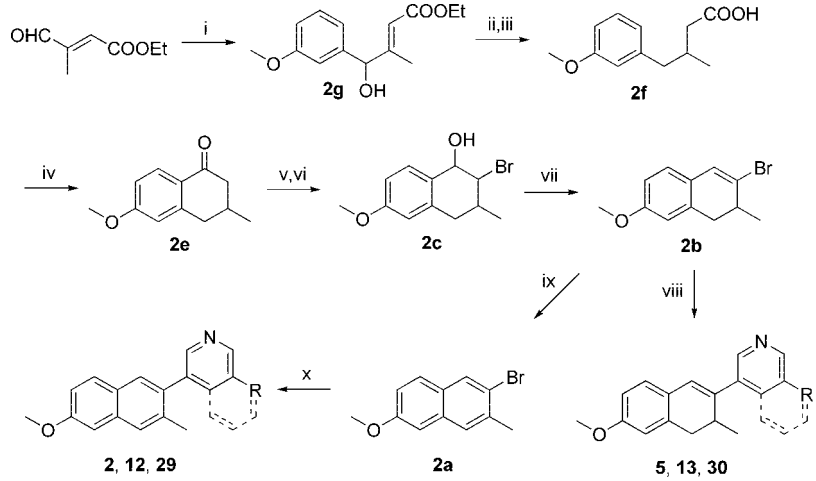
[a] Reagents and conditions: (i) $Zn(CN)_2$, $Pd(PPh_3)_4$, DMF, 100 °C; (ii) Tf_2NPh, KHMDS, THF/toluene, −78 °C; (iii) 3-pyridineboronic acid, $Pd(PPh_3)_4$, DMF, aq $NaHCO_3$, μw, 150 °C.

Biological Results

Inhibition of Human Adrenal Corticoid Producing CYP11B2 and CYP11B1 In Vitro (Table 1). The inhibitory activities of the compounds were determined in V79 MZh cells expressing either human CYP11B2 or CYP11B1.[10,36] The V79 MZh cells were incubated with [^{14}C]-deoxycorticosterone as substrate and the inhibitor at different concentrations. The product formation was monitored by HPTLC using a phosphoimager. Fadrozole, an aromatase (CYP19) inhibitor with proven ability to reduce corticoid formation in vitro and in vivo,[16] was used as a reference compound (CYP11B2, IC_{50} = 1 nM; CYP11B1, IC_{50} = 10 nM).

Most of the substituted pyridylnaphthalenes showed a high inhibitory activity at the target enzyme CYP11B2 with IC_{50} values in the low nanomolar range (Table 1). Some of the compounds displayed subnanomolar potency (IC_{50} < 1 nM) and turned out to be even stronger aldosterone synthase inhibitors than the reference substance fadrozole. The methoxyalkyl substituted compounds **22** and **26** exhibited IC_{50} values of 0.2 nM each. Hence, they are 5-fold more active than fadrozole (IC_{50} = 1 nM) and 30-fold more active than the unsubstituted parent compound **1** (IC_{50} = 6.2 nM). However, derivatization by polar and acidic residues in 5′-position resulted in a decrease in potency. This particularly applies to the carboxylic acids **15** and **19**, showing no or only low inhibitory activity and to a minor extent also to the phenolic compound **10** and the carboxamide **17**, with IC_{50} values of 94 nM each. Beside introduction of small residues in 4′- and 5′-position, an extension of the heterocyclic moiety by a condensed phenyl ring afforded the extraordinary potent isoquinoline compounds **28**−**30** with IC_{50} values in the range of 0.5−3.1 nM. Even the sterically demanding 5′-phenyl residue of compound **27** was still tolerated

Scheme 5[a]



[a] Reagents and conditions: (i) 3-methoxyphenylmagnesium bromide, THF, −5 °C; (ii) KOH, NaOH/water, reflux; (iii) H_2, Pd/C, AcOH, 60 °C; (iv) $(COCl)_2$, CH_2Cl_2, rt, then $AlCl_3$, CH_2Cl_2, −10 °C; (v) $CuBr_2$, ethyl acetate/$CHCl_3$, reflux; (vi) $NaBH_4$, methanol, 0 °C; (vii) *p*-toluenesulfonic acid, toluene, reflux; (viii) boronic acid, $Pd(OAc)_2$, TBAB, acetone, aq Na_2CO_3, μw, 150 °C; (ix) DDQ, toluene, reflux; (x) boronic acid, $Pd(PPh_3)_4$, DMF, aq $NaHCO_3$, μw, 150 °C.

Table 2. Inhibition of Selected Steroidogenic and Hepatic CYP Enzymes in Vitro

	% inhibition[a]		IC_{50} value[b] (nM)					
compd	CYP17[c]	CYP19[d]	CYP1A2[e,f]	CYP2B6[e,g]	CYP2C9[e,h]	CYP2C19[e,i]	CYP2D6[e,j]	CYP3A4[e,k]
9	42	47	1420	>50000	48970	45800	11100	21070
11	41	14	83	>25000	1888	>25000	>25000	1913
18	36	<5	488	>50000	>200000	>200000	>200000	9070
28	39	7	1619	16540	1270	3540	33110	3540

[a] Mean value of four experiments, standard deviation less than 10%. [b] Mean value of two experiments, standard deviation less than 5%. [c] *E. coli* expressing human CYP17; substrate progesterone, 25 μM; inhibitor concentration 2.0 μM; ketoconazole, IC_{50} = 2780 nM. [d] Human placental CYP19; substrate androstenedione, 500 nM, inhibitor concentration 500 nM; fadrozole, IC_{50} = 30 nM. [e] Recombinantly expressed enzymes from baculovirus-infected insect microsomes (Supersomes). [f] Furafylline, IC_{50} = 2419 nM. [g] Tranylcypromine, IC_{50} = 6240 nM. [h] Sulfaphenazole, IC_{50} = 318 nM. [i] Tranylcypromine, IC_{50} = 5950 nM. [j] Quinidine, IC_{50} = 14 nM. [k] Ketoconazole, IC_{50} = 57 nM.

(IC_{50} = 4.8 nM). In general, changing the carbocyclic core (naphthalene, 3-methyl- or dihydro-derivative) while simultaneously keeping the substitution pattern of the heterocycle had only little effect on the CYP11B2 inhibition as shown by the series **11**−**13** (IC_{50} = 1.2−4.2 nM) and **28**−**30** (IC_{50} = 0.5−3.1 nM). With regard to the inhibitory activity at the highly homologous CYP11B1, most of the tested compounds were less active than at CYP11B2. However, a noticeable inhibition with IC_{50} values in the range of 10−100 nM was observed in some cases. In particular, the 5′-methoxyalkylpyridine derivatives **22** (IC_{50} = 31 nM) and **26** (IC_{50} = 10 nM) as well as the methyl ester **16** (IC_{50} = 15 nM) turned out to be potent CYP11B1 inhibitors. Although introduction of substituents in the heterocyclic moiety mostly resulted in a moderate decrease in selectivity compared to the unsubstituted derivatives, the selectivity factors were still high for most of the tested compounds (factor 100−200). In case of 6-methoxy-3-methylnaphthalene **2**, the introduction of substituents in the heterocyclic moiety led to an enhanced selectivity. A methoxy substituent in 5′-position as accomplished in compound **12** increased the selectivity factor from 150 to 230 and the isoquinoline derivative **29** proved to be one of the most selective CYP11B2 inhibitors of the series with a selectivity factor of 272, thus being 27-fold more selective than fadrozole (selectivity factor = 10).

Inhibition of Hepatic and Steroidogenic CYP Enzymes (Tables 1 and 2). To further examine the influence of heteroaryl substitution on selectivity, the compounds were tested for inhibition of the hepatic CYP1A2 enzyme. CYP1A2 was strongly inhibited by all previous CYP11B2 inhibitors of the naphthalene and dihydronaphthalene type with unsubstituted heme-coordinating heterocycle, e.g., **1**−**4** exhibited more than 90% inhibition at an inhibitor concentration of 2 μM (Table 1). With regard to the potent CYP1A2 inhibitor **1**, derivatization of the heterocycle gave rise to compounds with a slightly reduced inhibitory potency, e.g., compounds **14**, **18**, **22**, and **25**, displaying approximately 80% inhibition. A pronounced decrease of CYP1A2 inhibition was observed in case of compounds **9**, **13**, and **29**−**30** (6−57%). However, the dihydronaphthalenes **6**, **13**, and **30** turned out to be chemically unstable and decomposition in DMSO solution was observed after storage at 2 °C (∼80% purity after three days), yielding considerable amounts of the aromatized analogues and traces of unidentified degradation products. Therefore, they were not taken into account for further biological evaluations despite their outstanding CYP1A2 selectivity. The CYP1A2 inhibition of some compounds was not determined at all due to either a low CYP11B2 potency (**15**, **17**, and **19**) or low CYP11B1 selectivity (**16**, **20**, **26**, and **27**).

For a set of four structurally diverse compounds (**9**, **11**, **18**, and **28**), an extended selectivity profile including inhibition of the steroidogenic enzymes CYP17 and CYP19 as well as inhibition of some crucial hepatic CYP enzymes (CYP2B6, CYP2C9, CYP2C19, CYP2D6, CYP3A4) was determined (Table 2). The inhibition of CYP17 was determined with the 50000*g* sediment of the *E. coli* homogenate recombinantly expressing human CYP17, progesterone (25 μM) as substrate, and the inhibitors at a concentration of 2 μM.[37] The tested compounds turned out to be moderately potent inhibitors of CYP17. The inhibition values ranked around 40%, corresponding with IC_{50} values of approximately 2000 nM or higher. The

Table 3. Pharmacokinetic Profile of Compounds **1**, **9**, and **28**

compd[a]	$t_{1/2\ z}$ (h)[b]	t_{max} (h)[c]	C_{max} (ng/mL)[d]	$AUC_{0-\infty}$ (ng·h/mL)[e]
1	5.4	1.0	222	1544
9	n.d.[f]	n.d.[f]	<1.5[g]	n.d.[f]
28	3.2	6.0	81	762
fadrozole	2.2	1.0	454	3575

[a] All compounds were applied perorally at a dose of 5 mg per kg body weight in four different cassette dosing experiments using male Wistar rats. [b] Terminal half-life. [c] Time of maximal concentration. [d] Maximal concentration. [e] Area under the curve. [f] n.d. = not detectable. [g] Below the limit of quantification.

inhibition of CYP19 at an inhibitor concentration of 500 nM was determined in vitro by use of human placental microsomes and [1β-^{3}H]androstenedione as substrate as described by Thompson and Siiteri[38] using our modification.[39] In this assay, no inhibition of CYP19 was observed for compounds **11**, **18**, and **28**. Only the amino-substituted compound **9** displayed a moderate inhibition of 47%. The IC_{50} values of the compounds for the inhibition of the hepatic CYP enzymes CYP1A2, CYP2B6, CYP2C9, CYP2C19, CYP2D6, and CYP3A4 were determined using recombinantly expressed enzymes from baculovirus-infected insect microsomes (Supersomes). The values of the CYP1A2 inhibition matched well the previously determined percental inhibition (Table 1). Methoxy compound **11** with 91% inhibition at 500 nM turned out to be a potent CYP1A2 inhibitor (IC_{50} = 83 nM), whereas the inhibitory potency decreased to 488 nM in the case of the ketone derivative **18**. A pronounced selectivity regarding the CYP1A2 inhibition was observed in case of compounds **9** and **28**, with IC_{50} values of approximately 1.5 μM. In most cases, the other investigated CYP enzymes were unaffected, e.g., IC_{50} values of **9** were greater than 10 μM versus CYP2B6, CYP2C9, CYP2C19, CYP2D6, and CYP3A4. (Table 2)

Pharmacokinetic Profile of Compounds 1, 9, and 28 (Table 3). The pharmacokinetic profile of compounds **9** and **28** was determined after peroral application to male Wistar rats and compared to the unsubstituted parent compound **1**. After administration of a 5 mg/kg dose in a cassette (N = 5), plasma samples were collected over 24 h and plasma concentrations were determined by HPLC-MS/MS. Fadrozole, which was used as a reference compound, displayed the highest plasma levels ($AUC_{0-\infty}$ = 3575 ng·h/mL), followed by **1** (1544 ng·h/mL) and **28** (762 ng·h/mL). At all sampling points, the amounts of **9** detected were found below the limit of quantification (1.5 ng per mL plasma). This experimental result may be either due to a fast metabolism of the aromatic amine or due to a lacking ability of this compound to permeate the cell membrane under physiological conditions. The half-lives were between 2.2−5.4 h, in which the elimination of fadrozole occurs faster than the elimination of the naphthalenes **1** and **28**. Compound **28** is slowly absorbed as indicated by the t_{max} of 6 h, whereas **1** is absorbed as fast as fadrozole (t_{max} = 1 h). Furthermore, no obvious sign of toxicity was noted in any animal over the duration of the experiment (24 h).

Discussion and Conclusion

The results obtained in the present study revealed that a variety of substituents in 4′- and 5′-position is tolerated with regard to the CYP11B2 potency. Most of the tested compounds were more potent than the unsubstituted parent compounds, and IC_{50} values less than 1 nM were observed in 7 cases (e.g., **22** and **26**, IC_{50} = 0.2 nM). Some of the compounds were also potent CYP11B1 inhibitors (e.g., **26**, IC_{50} = 10 nM). Interestingly, a precise relationship between the inhibition of CYP11B2 and CYP11B1 was observed: an increased or decreased inhibitory activity at the one enzyme was accompanied by an increased or decreased inhibitory activity at the other enzyme. For instance, based on the unsubstituted parent compound **1**, introduction of the methoxyalkyl substituent in compound **26** resulted in an enhanced inhibition of both CYP11B2 (IC_{50} = 0.2 nM) and CYP11B1 (IC_{50} = 10 nM), whereas introduction of the hydroxy group in compound **10** resulted in a decreased inhibitory potency at both CYP11B isoforms in a comparable order of magnitude (CYP11B2, IC_{50} = 94 nM; CYP11B1, IC_{50} = 8925 nM). This trend becomes particularly evident when plotting the CYP11B2 versus the CYP11B1 pIC_{50} values of the compounds presented in Table 1, revealing a reasonable linear correlation (r^2 = 0.86, n = 29). The finding that it is to some extent possible to change the inhibitory potency by the heteroaryl derivatization without significantly changing the selectivity versus either CYP11B2 or CYP11B1 is an indication that the inhibitor binding proceeds via similar protein−inhibitor interactions of the heterocyclic moiety with both CYP11B isoforms. Contrariwise, it has been shown earlier by us that variation of the carbocyclic skeleton instead of the heterocycle can significantly influence the selectivity. Therefore, no correlation is observed for a plot of the CYP11B2 and CYP11B1 pIC_{50} values of the naphthalenes[22] and dihydronaphthalenes[23] described previously by us that are functionalized with an unsubstituted 3-pyridine as heme complexing heterocycle (r^2 = 0.30, n = 20). Consistent with these findings, it can be assumed that both enzymes, CYP11B2 and CYP11B1, are structurally more diverse in the naphthalene binding site than in the heterocyclic binding site.

Interesting structure−activity relationships could also be observed with respect to electronic properties. Compounds bearing protic substituents in 5′-position rather poorly inhibited CYP11B2, whereas bioisosteric exchange by aprotic residues gave rise to highly potent aldosterone synthase inhibitors, e.g., the inhibitory potency increased by a factor of 40 from carboxamide **17** (IC_{50} = 94 nM) to the ethanone **18** (IC_{50} = 2.1 nM). A comparable increase of potency was observed when the protic hydroxy group was replaced by the aprotic methoxy group, e.g., the IC_{50} value decreased by a factor of 20 in case of compound **11** compared to the phenol **10** or by a factor of 40 in case of compound **22** compared to the primary alcohol **21**. Similarly, the methyl esters **16** and **20** were more active than the corresponding carboxylic acids **15** and **19**. However, a lack of membrane permeability must be taken into consideration as an alternative explanation. Figure 1 shows the molecular electrostatic potentials (MEP) mapped on the electron density surface of compounds **17**, **10**, and **21** and their bioisosteric analogues **18**, **11**, and **22**. Both the shape and the geometry of the compounds as well as the electrostatic potential distribution in the naphthalene moiety are very similar. In addition, all compounds contain a region in which the nitrogen of the pyridine ring presents a negative potential. However, areas with a distinct positive potential in the pyridine moiety are present in compounds **17**, **10**, and **21**, showing low inhibitory potency. In case of the highly potent bioisosters, these areas display less positive potential values with a more uniformly distributed electron charge. Hence, the difference in the electrostatic potential distribution is a reasonable explanation for the varying binding behavior within this set of compounds.

The heteroaryl derivatization had also a noticeable influence on the CYP1A2 potency of the compounds. Most of the substituted derivatives were still inhibiting CYP1A2 for more than 90% at a concentration of 2 μM. With respect to the compounds with a

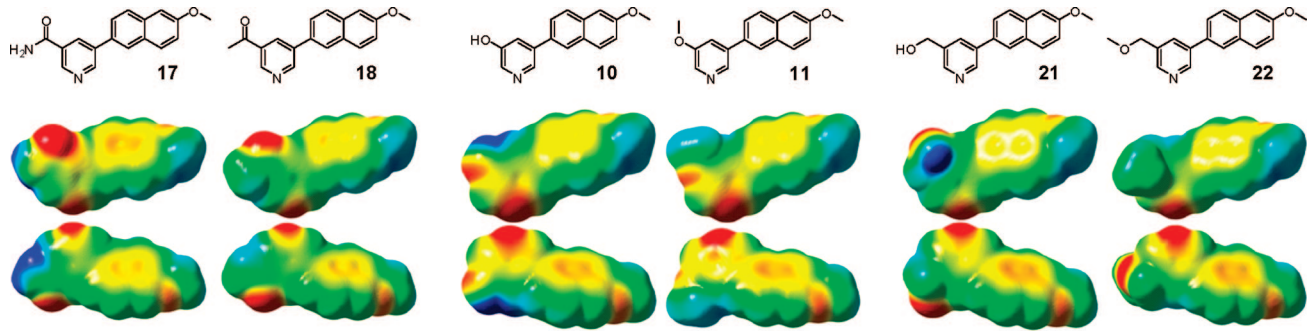


Figure 1. MEP of compounds **17**, **18**, **10**, **11**, **21**, and **22** (front and back view). The electrostatic potential surfaces were plotted with GaussView 3.0 in a range of −18.83 kcal/mol (red) to +21.96 kcal/mol (blue).

6-methoxynaphthalene core, a slight decrease to approximately 80% inhibition was observed in some cases. This effect was due to the introduction of substituents in 5′-position of the heterocycle. While no decrease of CYP1A2 inhibition was observed in the case of the rather small substituents in compounds **10**, **11**, and **21** (hydroxy, methoxy, and hydroxymethyl), a slight increase of the sterical bulk in compounds **14**, **18**, **22**, and **25** (ethoxy, acetyl, methoxymethyl, and hydroxymethyl) resulted in a decrease in CYP1A2 inhibition to 78−85%. On the other hand, some derivatives proved to be significantly less active with approximately 50% inhibition of CYP1A2, including the 4′-amino-substituted compound **9** and the isoquinoline **28** with IC_{50} values of 1420 and 1619 nM, respectively. The effect of changing 3-pyridine by 4-isoquinoline as heme-complexing heterocycle is particularly noteworthy. The three isoquinoline derivatives **28**, **29**, and **30** are considerably less active at CYP1A2 (6−57% inhibition) than their unsubstituted analogues **1**, **2**, and **5** (73−98% inhibition). An explanation might be found in the geometry of these molecules. The isoquinoline constrains the rotation around the carbon−carbon bond between the heterocycle and the naphthalene, especially in presence of the additional *ortho*-methyl groups in **29** and **30**. Thus, a coplanar conformation becomes energetically disfavored compared to the pyridine analogues and the sterically demanding heterocycle rotates out of the naphthalene plane. This loss of planarity is a reasonable explanation for the reduced inhibitory potency because both CYP1A2 substrates and inhibitors are usually small-volume molecules with a planar shape (e.g., caffeine[40] and furafylline[41]). An even more drastic effect on the CYP1A2 potency was observed in the case of the dihydronaphthalene type compounds. While the unsubstituted parent compound **5** exhibited 74% inhibition, introduction of the methoxy substituent in compound **13** led to a reduction to 18% and the isoquinoline derivative **30** displayed only 6% inhibition. The partly saturated core structure becomes flexible and disturbs the planarity. Factors other than steric might play an additional role for the decreased CYP1A2 inhibition, e.g., disturbed $\pi-\pi$ stacking contacts with aromatic amino acids in the CYP1A2 binding pocket due to the reduced number of aromatic carbons. Aromaticity has been identified to correlate positively with CYP1A2 inhibition in recent QSAR studies.[42] As dihydronaphthalenes **13** and **30** were found to be unstable in DMSO solution, the low potencies might be due to substance degradation. However, the decomposition (∼20% after three days) afforded mainly the aromatized naphthalene analogues, i.e., **12** and **29**, both displaying higher CYP1A2 inhibition than **13** and **30**.

In conclusion, we have shown that modifying the lead compounds **I** and **II** by introduction of substituents in the heterocyclic moiety has a clear effect on the activity and selectivity profile. Some substituents induced a significant increase in inhibitory potency versus CYP11B2. Compounds **22** and **26** with subnanomolar IC_{50} values are the most potent aldosterone synthase inhibitors so far. The undesirable high CYP1A2 inhibition that is present in the previously investigated derivatives could be overcome by certain residues, giving rise to compounds with an advantageous overall selectivity profile. It was also demonstrated that the naphthalene type aldosterone synthase inhibitors **1** and **28** were able to cross the gastrointestinal tract and reached the general circulation. Presently, the elucidated concepts are used to systematically modify other lead structures whereof some are under investigation for their ability to reduce aldosterone levels in vivo.

Experimental Section

Chemical and Analytical Methods. Melting points were measured on a Mettler FP1 melting point apparatus and are uncorrected. ^{1}H NMR and ^{13}C spectra were recorded on a Bruker DRX-500 instrument. Chemical shifts are given in parts per million (ppm), and tetramethylsilane (TMS) was used as internal standard for spectra obtained in DMSO-d_6 and $CDCl_3$. All coupling constants (J) are given in Hertz (Hz). Mass spectra (LC/MS) were measured on a TSQ Quantum (Thermo Electron Corporation) instrument with a RP18 100-3 column (Macherey Nagel) and with water/acetonitrile mixtures as eluents. Elemental analyses were carried out at the Department of Chemistry, University of Saarbrücken. Reagents were used as obtained from commercial suppliers without further purification. Solvents were distilled before use. Dry solvents were obtained by distillation from appropriate drying reagents and stored over molecular sieves. Flash chromatography was performed on silica gel 40 (35/40−63/70 μM) with hexane/ethyl acetate mixtures as eluents, and the reaction progress was determined by thin-layer chromatography analyses on Alugram SIL G/UV254 (Macherey Nagel). Visualization was accomplished with UV light and $KMnO_4$ solution. All microwave irradiation experiments were carried out in a CEM-Discover monomode microwave apparatus.

The following compounds were prepared according to previously described procedures: 6-methoxy-3-methyl-3,4-dihydronaphthalen-1(2*H*)-one (**2e**),[32] (2*E*)-4-hydroxy-4-(3-methoxyphenyl)-3-methyl-2-butenoic acid (**2g**),[32] 5-bromopyridin-3-ol (**10a**).[29]

Synthesis of the Target Compounds. Procedure A.[27] Pyridine boronic acid (0.75 mmol, 1 equiv), aryl bromide or -triflate (0.9−1.3 equiv), and tetrakis(triphenylphosphane)palladium(0) (43 mg, 37.5 μmol, 5 mol %) were suspended in 1.5 mL DMF in a 10 mL septum-capped tube containing a stirring magnet. To this was added a solution of $NaHCO_3$ (189 mg, 2.25 mmol, 3 equiv) in 1.5 mL water, and the vial was sealed with a Teflon cap. The mixture was irradiated with microwaves for 15 min at a temperature of 150 °C with an initial irradiation power of 100 W. After the reaction, the vial was cooled to 40 °C, the crude mixture was partitioned between ethyl acetate and water and the aqueous layer was extracted three times with ethyl acetate. The combined organic layers were dried over $MgSO_4$, and the solvents were removed in vacuo. The coupling

products were obtained after flash chromatography on silica gel (petroleum ether/ethyl acetate mixtures) and/or crystallization. If an oil was obtained, it was transferred into the hydrochloride salt by 1N HCl solution in diethyl ether.

Procedure B.[34] In a microwave tube, alkenyl bromide **7** (1 equiv), pyridine boronic acid (1.3 equiv), tetrabutylammonium bromide (1 equiv), sodium carbonate (3.5 equiv), and palladium acetate (1.5 mol %) were suspended in water/acetone 3.5/3 to give a 0.15 M solution of bromide **7** under an atmosphere of nitrogen. The septum-sealed vessel was irradiated under stirring and simultaneous cooling for 15 min at 150 °C with an initial irradiation power of 150 W. The reaction mixture was cooled to room temperature, diluted with a saturated ammonium chloride solution, and extracted several times with diethyl ether. The combined extracts were washed with brine, dried over $MgSO_4$, concentrated, and purified by flash chromatography on silica gel. The resulting oil was transferred into the hydrochloride salt by a 5−6 N HCl solution in 2-propanol and crystallized from ethanol.

Procedure C. To a suspension of NaH (1.15 equiv, 60% dispersion in oil) in 5 mL dry THF was added dropwise a solution of alcohol (1 equiv) in 5 mL THF at room temperature under an atmosphere of nitrogen. After hydrogen evolution ceased, a solution of methyliodide (3.3 equiv) in 5 mL THF was added dropwise, and the resulting mixture was stirred for 5 h at room temperature. The mixture was then treated with saturated aqueous NH_4Cl solution and extracted three times with ethyl acetate. The combined organic layers were washed with water and brine, dried over $MgSO_4$, and the solvent was evaporated in vacuo. The crude product was flash chromatographed on silica gel (petroleum ether/ethyl acetate mixtures) to afford the pure methylether. If an oil was obtained, it was transferred into the hydrochloride salt by 1N HCl solution in diethyl ether.

Procedure D. To a 0.05 M solution of carbonyl compound in dry methanol was added sodium borohydride (2 equiv). The reaction mixture was stirred for 1 h, diluted with diehtylether, and treated with saturated aqueous $NaHCO_3$ solution. The mixture was then extracted three times with ethyl acetate, washed twice with saturated aqueous $NaHCO_3$ solution and once with brine, and dried over $MgSO_4$. The filtrate was concentrated in vacuo, and the residue was filtered through a short pad of silica gel or flash chromatographed on silica gel (petroleum ether/ethyl acetate mixtures) to afford the corresponding alcohols.

3-(6-Methoxy-3-methylnaphthalen-2-yl)pyridine (2). The compound was obtained according to procedure A starting from **2a** (377 mg, 1.50 mmol) and 3-pyridineboronic acid (240 mg, 1.95 mmol) after flash chromatography on silica gel (petroleum ether/ethyl acetate, 4/1, $R_f = 0.16$) as a white solid (304 mg, 1.22 mmol, 81%), mp 106−107 °C. MS *m/z* 250.06 (MH^+). Anal. ($C_{17}H_{15}NO$) C, H, N.

3-(6-Methoxy-3-methyl-3,4-dihydronaphthalen-2-yl)pyridine (5). The compound was obtained according to procedure B starting from **2b** (127 mg, 0.50 mmol) and 3-pyridineboronic acid (80 mg, 0.65 mmol) after flash chromatography on silica gel (petroleum ether/ethyl acetate, 4/1, $R_f = 0.20$), precipitation as HCl salt, and crystallization from ethanol as a white solid (50 mg, 0.17 mmol, 35%), mp (HCl salt) 186−187 °C. MS *m/z* 252.02 (MH^+). Anal. ($C_{17}H_{17}NO \cdot HCl \cdot 0.2H_2O$) C, H, N.

6-(Pyridin-3-yl)-7,8-dihydronaphthalene-2-carbonitrile (6). The copound was prepared according to procedure A starting from 3-pyridineboronic acid (107 mg, 0.87 mmol) and **6a** (189 mg, 0.62 mmol). After flash chromatography on silica gel (petroleum ether/ethyl acetate, 2/1, $R_f = 0.10$) pure **6** was obtained as a white, crystalline solid (100 mg, 0.43 mmol, 69%). Treatment with hydrochloride acid (0.1 N in Et_2O) yielded the hydrochloride salt of **6** (110 mg, 0.41 mmol, 66%) as a white solid, mp (HCl salt) 264−268 °C. MS *m/z* 223.23 (MH^+). Anal. ($C_{16}H_{12}N_2 \cdot HCl \cdot 0.4H_2O$) C, H, N.

3-(6-Methoxynaphthalen-2-yl)-4-methylpyridine (7). The compound was prepared according to procedure A starting from 6-methoxy-2-naphthaleneboronic acid (131 mg, 0.65 mmol) and 3-bromo-4-methylpyridine (86 mg, 0.50 mmol). After flash chromatography on silica gel (petroleum ether/ethyl acetate, 7/3, $R_f = 0.10$) pure **7** was obtained as a white solid (65 mg, 0.26 mmol, 52%), mp (HCl salt) 172−174 °C. MS *m/z* 250.30 (MH^+). Anal. ($C_{17}H_{15}NO \cdot HCl \cdot 0.1H_2O$) C, H, N.

6-(4-Methylpyridin-3-yl)-2-naphthonitrile (8). Triflate **8a** (151 mg, 0.50 mmol), 4-methyl-3-pyridineboronic acid (89 mg, 0.65 mmol), K_2CO_3 (138 mg, 1.0 mmol), and $Pd(dppf)Cl_2$ (37 mg, 0.05 mmol) were suspended in 4.0 mL of a 4:4:1 mixture of toluene/acetone/water. This mixture was heated to 125 °C by microwave irradiation for 15 min (initial irradiation power 150 W). After cooling to room temperature, 15 mL of distilled water were added and the reaction mixture was extracted four times with 10 mL of Et_2O. After washing the combined organic fractions with water (twice) and brine, drying over $MgSO_4$, and evaporation of the solvent crude product, **8** was obtained as a yellow solid (127 mg). Further purification by flash chromatography on silica gel (petroleum ether/ethyl acetate, 2/5, $R_f = 0.20$) and subsequent crystallization of the free base as hydrochloride salt gave 52 mg (0.19 mmol, 37%) of pure **8** (HCl salt) as an yellowish solid, mp (HCl salt) decomposition above 210 °C. MS *m/z* 245.30 (MH^+). Anal. ($C_{17}H_{12}N_2 \cdot HCl \cdot 0.5H_2O$) C, H, N.

3-(6-Methoxynaphthalen-2-yl)pyridin-4-amine (9). The compound was prepared according to procedure A starting from 6-methoxy-2-naphthaleneboronic acid (131 mg, 0.65 mmol) and 3-bromopyridin-4-amine (86 mg, 0.50 mmol). After crystallization from acetone, pure **9** was obtained as a white solid (39 mg, 0.16 mmol, 31%), mp 155−156 °C. MS *m/z* 251.28 (MH^+). Anal. ($C_{16}H_{14}N_2O$) C, H, N.

5-(6-Methoxynaphthalen-2-yl)pyridin-3-ol (10). The compound was prepared according to procedure A starting from 6-methoxy-2-naphthaleneboronic acid (131 mg, 0.65 mmol) and **10a** (87 mg, 0.50 mmol). After crystallization from acetone/diethyl ether, pure **10** was obtained as an off-white solid (86 mg, 0.34 mmol, 68%), mp 172−175 °C. MS *m/z* 252.02 (MH^+). Anal. ($C_{16}H_{13}NO_2 \cdot 0.7H_2O$) C, H, N: calcd, 5.31; found, 5.79.

3-Methoxy-5-(6-methoxynaphthalen-2-yl)pyridine (11). The compound was prepared according to procedure A starting from 6-methoxy-2-naphthaleneboronic acid (131 mg, 0.65 mmol) and 3-bromo-5-methoxypyridine (94 mg, 0.50 mmol). After flash chromatography on silica gel (petroleum ether/ethyl acetate, 7/3, $R_f = 0.10$), pure **11** was obtained as a white solid (80 mg, 0.30 mmol, 60%), mp (HCl salt) 211−214 °C. ^1H NMR (500 MHz, CD_3OD): δ 3.96 (s, 3H), 4.15 (s, 3H), 7.23 (dd, $^3J = 9.1$ Hz, $^4J = 2.5$ Hz, 1H), 7.32 (d, $^4J = 2.2$ Hz, 1H), 7.85 (dd, $^3J = 8.5$ Hz, $^4J = 1.9$ Hz, 1H), 7.92 (d, $^3J = 8.8$ Hz, 1H), 7.97 (d, $^3J = 8.5$ Hz, 1H), 8.29 (d, $^4J = 1.5$ Hz, 1H), 8.52 (s, 1H), 8.54 (s, 1H), 8.86 (s, 1H). ^{13}C NMR (125 MHz, CD_3OD): δ 57.9, 58.3, 108.5, 120.9, 121.9, 128.1, 128.5, 130.2, 131.4, 132.5, 134.5, 136.7, 138.9, 139.1, 142.6, 158.4, 160.4. MS *m/z* 266.26 (MH^+). Anal. ($C_{17}H_{15}NO_2 \cdot HCl \cdot 0.3H_2O$) C, H, N.

3-Methoxy-5-(6-methoxy-3-methylnaphthalen-2-yl)pyridine (12). The compound was obtained according to procedure A starting from **2a** (377 mg, 1.50 mmol) and 5-methoxy-3-pyridineboronic acid (298 mg, 1.95 mmol) after flash chromatography on silica gel (petroleum ether/ethyl acetate, 3/1, $R_f = 0.16$) as a white solid (328 mg, 1.17 mmol, 78%), mp 106−107 °C. ^1H NMR (500 MHz, $CDCl_3$): δ 2.40 (s, 3H), 3.91 (s, 3H), 3.94 (s, 3H), 7.12 (m, 2H), 7.23 (dd, $^4J = 2.8$ Hz, $^4J = 1.9$ Hz, 1H), 7.62 (s, 1H), 7.64 (s, 1H), 7.71 (d, $^3J = 8.8$ Hz, 1H), 8.28 (d, $^4J = 1.9$ Hz, 1H), 8.33 (d, $^4J = 2.8$ Hz, 1H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 21.0, 55.3, 55.6, 104.9, 118.6, 121.5, 127.4, 127.5, 128.6, 129.2, 134.1, 134.4, 134.5, 135.8, 138.0, 142.6, 155.2, 158.1. MS *m/z* 280.08 (MH^+). Anal. ($C_{18}H_{17}NO_2$) C, H, N.

3-Methoxy-5-(6-methoxy-3-methyl-3,4-dihydronaphthalen-2-yl)pyridine (13). The compound was obtained according to procedure B starting from **2b** (253 mg, 1.00 mmol) and 5-methoxy-3-pyridineboronic acid (199 mg, 1.30 mmol) after flash chromatography on silica gel (petroleum ether/ethyl acetate, 4/1, $R_f = 0.14$), precipitation as HCl salt, and crystallization from ethanol as a yellow solid (84 mg, 0.26 mmol, 26%), mp (HCl salt) 181−182 °C. ^1H NMR (500 MHz, CD_3OD): δ 0.94 (d, $^3J = 7.0$ Hz, 3H),

2.71 (dd, ^2J = 15.3 Hz, ^3J = 1.2 Hz, 1H), 3.02 (m, 1H), 3.13 (dd, ^2J = 15.5 Hz, ^3J = 6.4 Hz, 1H), 3.74 (s, 3H), 4.01 (s, 3H), 6.72 (m, 2H), 7.15 (d, ^3J = 8.2 Hz, 1H), 7.19 (s, 1H), 8.22 (m, 1H), 8.34 (d, ^4J = 2.4 Hz, 1H), 8.59 (d, ^4J = 1.5 Hz, 1H). ^{13}C NMR (125 MHz, CD_3OD): δ 17.8, 30.6, 36.7, 55.8, 57.9, 112.9, 115.6, 116.6, 127.0, 127.2, 127.8, 129.8, 130.3, 132.3, 137.1, 137.2, 150.9, 160.1. MS *m/z* 281.96 (MH^+). Anal. ($C_{18}H_{19}NO_2 \cdot HCl \cdot 0.2H_2O$) C, H, N.

3-Ethoxy-5-(6-methoxynaphthalen-2-yl)pyridine (14). The compound was prepared according to procedure A starting from 6-methoxy-2-naphthaleneboronic acid (131 mg, 0.65 mmol) and **14a** (101 mg, 0.50 mmol). After crystallization from ethyl acetate/petroleum ether, pure **14** was obtained as a white solid (33 mg, 0.17 mmol, 23%), mp decomposition above 130 °C. MS *m/z* 280.05 (MH^+). Anal. ($C_{18}H_{17}NO_2 \cdot 0.2H_2O$) C, H, N.

5-(6-Methoxynaphthalen-2-yl)pyridine-3-carboxylic acid (15). The compound was prepared according to procedure A starting from 6-methoxy-2-naphthaleneboronic acid (131 mg, 0.65 mmol) and 5-bromonicotinic acid (101 mg, 0.50 mmol). After crystallization from methanol/water, pure **15** was obtained as an off-white solid (74 mg, 0.26 mmol, 53%), mp decomposition above 300 °C. MS *m/z* 279.98 (MH^+). Anal. ($C_{17}H_{13}NO_3 \cdot HCl \cdot 0.3H_2O$) C, H, N.

Methyl 5-(6-methoxynaphthalen-2-yl)pyridine-3-carboxylate (16). Carboxylic acid **15** (45 mg, 0.16 mmol) was dissolved in 20 mL dry methanol and 0.05 mL concentrated H_2SO_4 (98%) was added. The whole mixture was refluxed for 10 h, and thereafter the excess methanol was distilled off. The residue was taken up in 50 mL of ethyl acetate, and the organic layer was washed several times with 5% aqueous Na_2CO_3 solution, water, and brine. After drying over $MgSO_4$, the solvent was evaporated in vacuo. After flash chromatography on silica gel (petroleum ether/ethyl acetate, 7/3, R_f = 0.34), pure **16** was obtained as an off-white solid (28 mg, 0.10 mmol, 60%), mp 150−151 °C. MS *m/z* 293.97 (MH^+). Anal. ($C_{18}H_{14}NO_3$) C, H, N.

5-(6-Methoxynaphthalen-2-yl)pyridine-3-carboxamide (17). The compound was prepared according to procedure A starting from 6-methoxy-2-naphthaleneboronic acid (131 mg, 0.65 mmol) and 5-bromonicotinamide (92 mg, 0.50 mmol). After crystallization from acetone/diethyl ether, pure **17** was obtained as a white solid (55 mg, 0.20 mmol, 40%), mp 245−247 °C. MS *m/z* 279.07 (MH^+). Anal. ($C_{17}H_{14}N_2O_2$) C, H, N.

1-[5-(6-Methoxynaphthalen-2-yl)pyridin-3-yl]ethanone (18). The compound was prepared according to procedure A starting from 6-methoxy-2-naphthaleneboronic acid (131 mg, 0.65 mmol) and 3-acetyl-5-bromopyridine (100 mg, 0.50 mmol). After crystallization from acetone/diethyl ether, pure **18** was obtained as a white solid (75 mg, 0.27 mmol, 54%), mp 159−160 °C. MS *m/z* 278.09 (MH^+). Anal. ($C_{18}H_{15}NO_2 \cdot 0.1H_2O$) C, H, N.

[5-(6-Methoxynaphthalen-2-yl)pyridin-3-yl]acetic acid (19). The compound was prepared according to procedure A starting from 6-methoxy-2-naphthaleneboronic acid (131 mg, 0.65 mmol) and 5-bromo-3-pyridineacetic acid (108 mg, 0.50 mmol). After crystallization from methanol/water, pure **19** was obtained as a white solid (70 mg, 0.24 mmol, 48%), mp decomposition above 210 °C. MS *m/z* 293.97 (MH^+). Anal. ($C_{18}H_{15}NO_3 \cdot 0.5H_2O$) C, H, N.

Methyl [5-(6-methoxynaphthalen-2-yl)pyridin-3-yl]acetate (20). The compound was prepared as described for **16** starting from **19** (145 mg, 0.49 mmol). After flash chromatography on silica gel (petroleum ether/ethyl acetate, 1/1, R_f = 0.18), pure **20** was obtained as a white solid (81 mg, 0.26 mmol, 53%), mp 145−146 °C. MS *m/z* 308.04 (MH^+). Anal. ($C_{19}H_{17}NO_3$) C: calcd, 74.25, found, 74.72, H, N.

[5-(6-Methoxynaphthalen-2-yl)pyridin-3-yl]methanol (21). The compound was prepared according to procedure A starting from 6-methoxy-2-naphthaleneboronic acid (131 mg, 0.65 mmol) and **21a** (94 mg, 0.50 mmol). After crystallization from acetone/diethyl ether, pure **21** was obtained as a white solid (86 mg, 0.32 mmol, 65%), mp 193−194 °C. MS *m/z* 266.05 (MH^+). Anal. ($C_{17}H_{15}NO_2 \cdot 0.1H_2O$) C, H, N.

3-(Methoxymethyl)-5-(6-methoxynaphthalen-2-yl)pyridine (22). The compound was prepared according to procedure C starting from **21** (150 mg, 0.57 mmol) using methyl iodide (82 μL, 1.32 mmol). After flash chromatography on silica gel (petroleum ether/ethyl acetate, 1/1, R_f = 0.22), pure **22** was obtained as an off-white solid (80 mg, 0.29 mmol, 50%), mp 121−122 °C. MS *m/z* 279.91 (MH^+). Anal. ($C_{18}H_{17}NO_2$) C, H, N.

[4-(6-Methoxynaphthalen-2-yl)pyridin-3-yl]methanol (23). The compound was prepared according to procedure A starting from 6-methoxy-2-naphthaleneboronic acid (131 mg, 0.65 mmol) and **23a** (94 mg, 0.50 mmol). After crystallization from acetone/diethyl ether, pure **23** was obtained as a white solid (90 mg, 0.34 mmol, 68%), mp decomposition above 240 °C. MS *m/z* 266.05 (MH^+). Anal. ($C_{17}H_{15}NO_2 \cdot 0.2H_2O$) C, H, N.

4-(Methoxymethyl)-3-(6-methoxynaphthalen-2-yl)pyridine (24). The compound was prepared according to procedure C starting from **23** (150 mg, 0.57 mmol) using methyl iodide (82 μL, 1.32 mmol). After flash chromatography on silica gel (petroleum ether/ethyl acetate, 1/1, R_f = 0.23), pure **24** was obtained as an off-white solid (74 mg, 0.26 mmol, 46%), mp (HCl salt) 174−177 °C. MS *m/z* 279.91 (MH^+). Anal. ($C_{18}H_{17}NO_2 \cdot 0.2H_2O$) C, H, N.

1-[5-(6-Methoxynaphthalen-2-yl)-pyridin-3-yl]ethanol (25). The compound was prepared according to procedure D starting from **18** (50 mg, 0.18 mmol) using $NaBH_4$ (8.0 mg, 0.21 mmol). After flash chromatography on silica gel (petroleum ether/ethyl acetate, 1/1, R_f = 0.24), pure **25** was obtained as a white solid (28 mg, 0.10 mmol, 56%), mp 154−155 °C. MS *m/z* 280.05 (MH^+). Anal. ($C_{18}H_{17}NO_2$) C, H, N.

3-(1-Methoxyethyl)-5-(6-methoxynaphthalen-2-yl)pyridine (26). The compound was prepared according to procedure C starting from **25** (70 mg, 0.25 mmol) using methyl iodide (41 μL, 0.66 mmol). After flash chromatography on silica gel (petroleum ether/ethyl acetate, 1/1, R_f = 0.23), pure **26** was obtained as a yellowish solid (26 mg, 0.08 mmol, 35%), mp 124−125 °C. MS *m/z* 294.11 (MH^+). Anal. ($C_{19}H_{19}NO_2$) C, H, N.

3-(6-Methoxynaphthalen-2-yl)-5-phenylpyridine (27). The compound was prepared according to procedure A starting from 6-methoxy-2-naphthaleneboronic acid (394 mg, 1.95 mmol) and 3-bromo-5-phenylpyridine (351 mg, 1.50 mmol). After flash chromatography on silica gel (petroleum ether/ethyl acetate, 2/1, R_f = 0.23), pure **27** was obtained as a white, crystalline solid (455 mg, 1.46 mmol, 97%), mp 216−217 °C. MS *m/z* 312.09 (MH^+). Anal. ($C_{22}H_{17}NO \cdot 0.4H_2O$) C, H, N.

4-(6-Methoxynaphthalen-2-yl)isoquinoline (28). The compound was prepared according to procedure A starting from 6-methoxy-2-naphthaleneboronic acid (131 mg, 0.65 mmol) and 4-bromoisoquinoline (104 mg, 0.50 mmol). After flash chromatography on silica gel (petroleum ether/ethyl acetate, 7/3, R_f = 0.21), pure **28** was obtained as a white solid (44 mg, 0.16 mmol, 31%), mp 185−186 °C. MS *m/z* 286.07 (MH^+). Anal. ($C_{20}H_{15}NO \cdot 0.1H_2O$) C, H, N.

4-(6-Methoxy-3-methylnaphthalen-2-yl)isoquinoline (29). The compound was obtained according to procedure A starting from **2a** (377 mg, 1.50 mmol) and 4-isoquinolineboronic acid (337 mg, 1.95 mmol) after flash chromatography on silica gel (dichloromethane/methanol 99/1, R_f = 0.26) as yellow oil, which solidified with diethyl ether as a pale-yellow solid (178 mg, 0.59 mmol, 40%), mp 156−157 °C. MS *m/z* 300.10 (MH^+). Anal. ($C_{21}H_{11}NO$) C, H, N.

4-(6-Methoxy-3-methyl-3,4-dihydronaphthalen-2-yl)isoquinoline (30). The compound was obtained according to procedure B starting from **2b** (253 mg, 1.00 mmol) and 4-isoquinolineboronic acid (225 mg, 1.30 mmol) after two flash chromatographical separations on silica gel (petroleum ether/ethyl acetate, 5/1, R_f = 0.20, and dichloromethane/methanol 99/1, R_f = 0.27) and precipitation as HCl salt as a yellow solid (112 mg, 0.33 mmol, 17%), mp 149−150 °C. MS *m/z* 302.18 (MH^+). Anal. ($C_{21}H_{19}NO \cdot HCl \cdot 0.2H_2O$) C, H, N.

Biological Methods. (1) *Enzyme preparations*. CYP17 and CYP19 preparations were obtained by described methods: the 50000*g* sediment of *E. coli* expressing human CYP17[37] and

microsomes from human placenta for CYP19.[39] (2) *Enzyme assays*. The following enzyme assays were performed as previously described: CY17[37] and CYP19.[39] (3) *Activity and selectivity assay using V79 cells*. V79 MZh 11B1 and V79 MZh 11B2 cells[36] were incubated with [4-^{14}C]-11-deoxycorticosterone as substrate and inhibitor in at least three different concentrations. The enzyme reactions were stopped by addition of ethyl acetate. After vigorous shaking and a centrifugation step (10000*g*, 2 min), the steroids were extracted into the organic phase, which was then separated. The conversion of the substrate was analyzed by HPTLC, and a phosphoimaging system as described.[10,22] (4) *Inhibition of human hepatic CYP enzymes*. The recombinantly expressed enzymes from baculovirus-infected insect microsomes (Supersomes) were used and the manufacturer's instructions (www.gentest.com) were followed. (5) *In vivo pharmacokinetics*. Animal trials were conducted in accordance with institutional and international ethical guidelines for the use of laboratory animals. Male Wistar rats weighing 317−322 g (Janvier, France) were housed in a temperature-controlled room (20−22 °C) and maintained in a 12 h light/12 h dark cycle. Food and water were available ad libitum. The animals were anaesthetised with a ketamine (135 mg/kg)/xylazine (10 mg/kg) mixture, and cannulated with silicone tubing via the right jugular vein. Prior to the first blood sampling, animals were connected to a counterbalanced system and tubing to perform blood sampling in the freely moving rat. Separate stock solutions (5 mg/mL) were prepared for the tested compounds in Labrasol/water (1:1; v/v), leading to a clear solution. Immediately before application, the cassette dosing mixture was prepared by adding equal volumes of the five stock solutions to end up with a final concentration of 1 mg/mL for each compound. The mixture was applied perorally to 3 rats with an injection volume of 5 mL/kg (time 0). Then 400 μL of blood were taken via jugularis catheter 1 h before application and then 1 and 2 h after application. Immediately, equal volume (400 μL) of 0.9% NaCl (37 °C) was reinjected intravenously to keep the blood volume stable. Then 4, 6, 8, 10 and 24 h after application, 250 μL of blood were sampled without balancing the blood volume. Blood samples were centrifuged at 3000*g* for 10 min at 4 °C. Plasma was harvested and kept at −20 °C until analysis. The mean of absolute plasma concentrations (±SEM) was calculated for the three rats, and the regression was performed on group mean values. The pharmacokinetic analysis was performed using a noncompartment model (PK Solutions 2.0, Summit Research Services). HPLC-MS/MS analysis and quantification of the samples was carried out on a Surveyor-HPLC-system coupled with a TSQ Quantum (ThermoFinnigan) triple quadrupole mass spectrometer equipped with an electrospray interface (ESI).

Computational Methods. MEP. For each docked compound, geometry optimization was performed at the B3LYP/6-31G* density functional levels by means of the Gaussian03 software, and the molecular electrostatic potential (MEP) maps were plotted using GaussView3, the 3-D molecular graphics package of Gaussian.[43] These electrostatic potential surfaces were generated by mapping 6-31G* electrostatic potentials onto surfaces of molecular electron density (isovalue = 0.002 electron/Å).[44]

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Supporting Information Available: Individual plasma levels of each compound and animal, graphs and equations of the linear regression of pIC_{50} values, NMR-spectroscopic data of compounds **2**, **5**−**10**, **14**−**30**, full experimental details and spectroscopic characterization of the reaction intermediates **2a**−**2d**, **2f**, **6a**, **6b**, **8a**, **14a**, **21a**, **23a**, elemental analysis results of compounds **2**, **5**−**30**. This material is available free of charge via the Internet at http://pubs.acs.org.

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