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Replacement of Imidazolyl by Pyridyl in Biphenylmethylenes Results in Selective CYP17 and Dual CYP17/CYP11B1 Inhibitors for the Treatment of Prostate Cancer

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Androgens are well-known to stimulate prostate cancer (PC) growth. Thus, blockade of androgen production in testes and adrenals by CYP17 inhibition is a promising strategy for the treatment of PC. Moreover, many PC patients suffer from glucocorticoid overproduction, and importantly mutated androgen receptors can be stimulated by glucocorticoids. In this study, the first dual inhibitor of CYP17 and CYP11B1 (the enzyme responsible for the last step in glucocorticoid biosynthesis) is described. A series of biphenylmethylene pyridines has been designed, synthesized, and tested as CYP17 and CYP11B1 inhibitors. The most active compounds were also tested for selectivity against CYP11B2 (aldosterone synthase), CYP19 (aromatase), and hepatic CYP3A4. In detail, compound **6** was identified as a dual inhibitor of CYP17/CYP11B1 (IC_{50} values of 226 and 287 nM) showing little inhibition of the other enzymes as well as compound **9** as a selective, highly potent CYP17 inhibitor (IC_{50} = 52 nM) exceeding abiraterone in terms of activity and selectivity.

Introduction

As the growth of up to 80% of prostate carcinoma is androgen dependent,[1] blockage of androgen production or action will effectively prevent cancer cells from proliferation. Currently, the standard therapy for prostate tumor is the so-called "combined androgen blockade" (CAB[a]), which means orchidectomy or treatment with gonadotropin-releasing hormone (GnRH) analogues (chemical castration)[2] combined with androgen receptor antagonists.[3] In CAB therapy, orchidectomy or GnRH treatment is employed to annihilate testicular androgen production and consequently to reduce plasma androgen concentration. However, although about 90% of androgens are no longer produced, the minor amount of androgens from the adrenals is still sufficient to prompt cancer growth. To solve this problem, androgen receptor antagonists are additionally applied to prevent androgen stimulation. Nonetheless, mutations in androgen receptor are the reason for the emerging resistance to this therapy. The mutated receptor can be activated by recognizing antiandrogens[4] and glucocorticoids[5] as agonists.

A promising alternative to CAB is the total blockage of androgen biosynthesis in both testes and adrenals. An elegant way to achieve this is to inhibit 17α-hydroxylase-17,20-lyase (CYP17). CYP17 is the crucial enzyme catalyzing the conversion of pregnenolone and progesterone to dehydroepiandrosterone (DHEA) and androstenedione in gonadal and adrenal glands[6] (Chart 1). Subsequently, these two weak androgens are converted to testosterone. In the prostate cells, the latter steroid is transformed into the most potent androgen dihydrotestosterone (DHT) by 5α-reductase (5α-R). Because inhibitors of 5α-R[7] reduce the intracellular prostatic DHT stimulation, they are exploited in the treatment of benign prostatic hyperplasia. For PC treatment, however, total blockade of androgen production is necessary.

However, a major portion of patients becomes "castration resistant", which might be caused by androgen receptor mutations, as mentioned above. Androgen receptor activation by glucocorticoids, especially cortisol, stimulates cancer cell proliferation.[5] Moreover, small cell anaplastic prostate carcinoma, which originates from neuroendocrine cells, is found to be capable of ectopic ACTH production.[8a] The elevated ACTH levels promote adrenals to synthesize and release high concentrations of cortisol leading to Cushing's syndrome,[8b,c,d] diabetes mellitus, osteoporosis, hypertension, and obesity. High cortisol levels have also been considered as a sign of PC cell growth.[8b] Importantly, some patients die of severe infections largely due to the immunosuppression caused by the glucocorticoid.[8c,d] Hence, for these patients the control of cortisol concentration is urgently needed. As the key step in the biosynthesis of this hormon is catalyzed by CYP11B1 (Chart 1), additional inhibition of this enzyme could be a substantial way to improve curative effects, relieve symptoms, and increase survival of prostate cancer patients.

Two decades ago, the first attempt to cure prostate cancer via CYP17 inhibition was described: the antimycotic ketoconazole (Chart 2) was exploited as CYP17 inhibitor for the treatment of prostate cancer clinically.[9a] Although curative

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[a]Abbreviations: PC, prostate cancer; CYP, cytochrome P450; CAB, combined androgen blockade; GnRH, gonadotropin-releasing hormone; CYP17, 17α-hydroxylase-17,20-lyase; CYP11B1, 11β- hydroxylase; CPY19, aromatase, estrogen synthase; CYP11B2, aldosterone synthase; DHEA, dehydroepiandrosterone; DHT, dihydrotestosterone; DOC, 11-deoxycorticosterone; 5α-R, 5α-reductase, TBAB, tetrabutylammonium bromide; DCM, dichloromethane.

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Chart 1. Androgen Biosynthesis

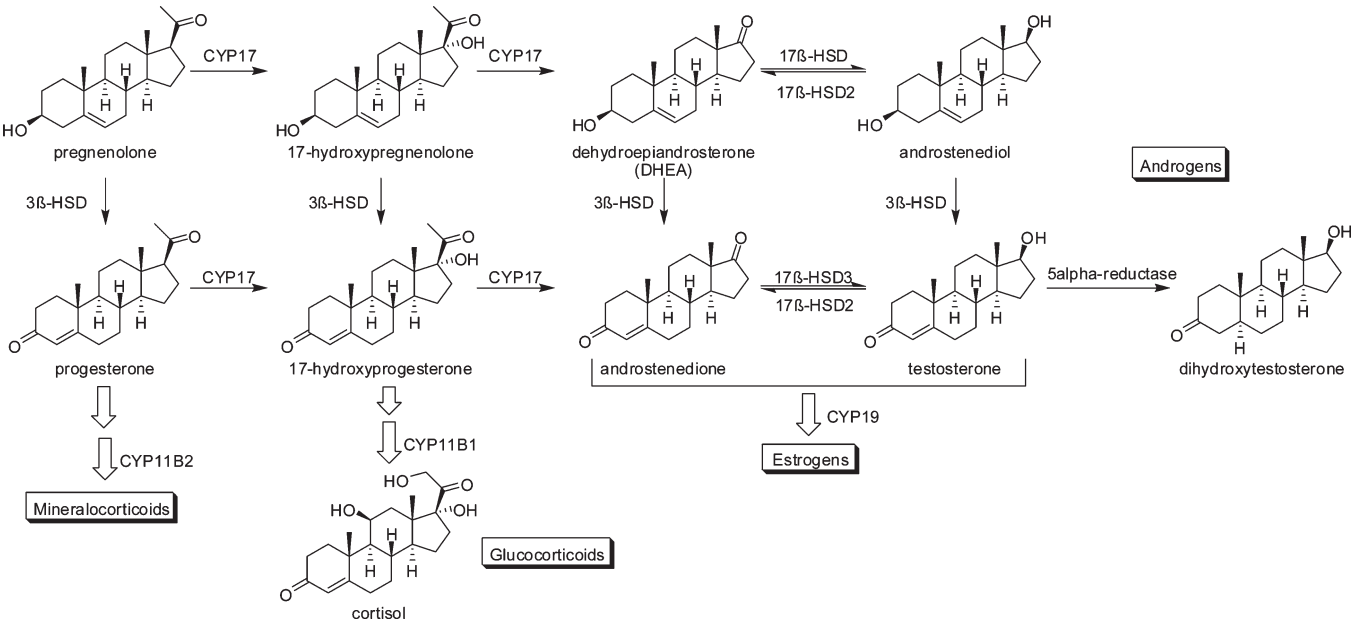
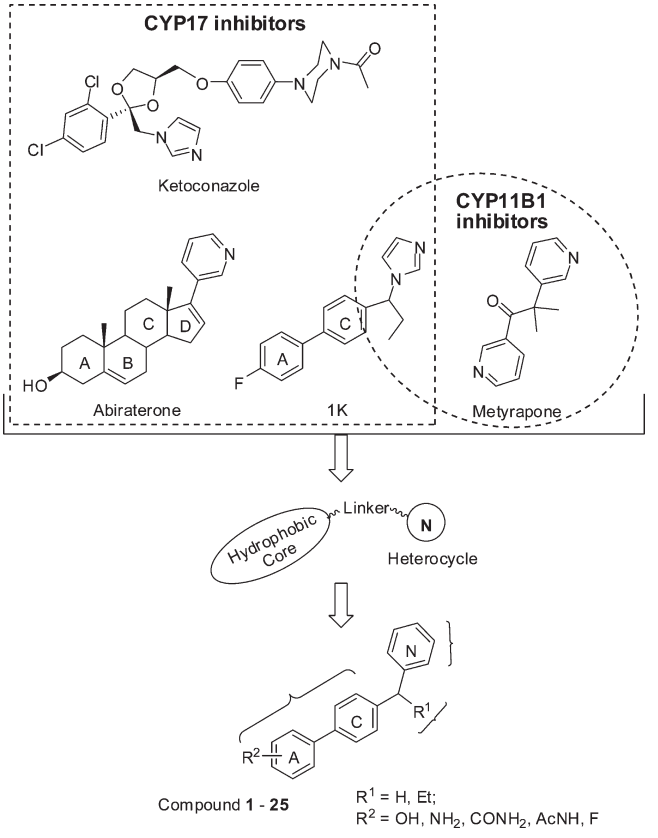


Chart 2. Inhibitor Design Conception

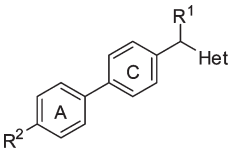


response was observed,[9b] ketoconazole was withdrawn because of side effects associated with its poor selectivity against other steroidogenic and hepatic CYP enzymes. Later, several steroidal inhibitors were designed on the basis of endogenous substrates.[10] Recently, abiraterone[11a] (Chart 2) entered into phase II and phase III clinical trials. Castration resistant patients showed good response to abiraterone,[11b] which confirms the superiority of prostate carcinoma treatment via CYP17 inhibition. However, the potential side effects might not be neglected which are caused by the affinity of steroidal scaffolds toward steroid receptors. Therefore, nonsteroidal CYP17 inhibitors, such as heterocycle substituted tetrahydronaphthalenes,[12a] adamantine carboxylates,[12b] and biphenyls,[13] attract more and more attention.

Our group has reported about several series of biphenyl methylene imidazoles[13] as potent CYP17 inhibitors. All these compounds were designed on the basis of the mechanism that the sp^2 hybrid nitrogen can coordinate with the heme iron, which was first[14a] identified for aromatase (CYP19, estrogen synthase) inhibitors[14] and later was also proven to be valid for aldosterone synthase (CYP11B2)[15] and CYP17 inhibitors.[10,12,13]

Although potent inhibitors, like **1K**[13e] (Chart 2, IC_{50} = 131 nM; for comparison, abiraterone IC_{50} = 72 nM), were obtained after systematic modification on the biphenyl core and methylene bridge, activity and selectivity could possibly be further improved. Furthermore, compounds with additional CYP11B1 inhibition seemed feasible. After abiraterone, **1K**, and metyrapone (Chart 2) were superimposed using their heterocyclic N as a common atom, it is apparent that all of three compounds are composed of three structural features: the hydrophobic core, an alkyl linker in between, and a heterocycle containing N. Since metyrapone is a CYP11B1 inhibitor, which has been applied in the clinic to treat Cushing's syndrome for nearly half a century,[16] and **1K** also exhibits weak CYP11B1 inhibition, this common structural pattern might be the basis of dual inhibition while the inhibitory potency toward each enzyme depends on the choice of the structural features. It is obvious that the steroidal scaffold of abiraterone and the biphenyl core of **1K** mate sterically. Nevertheless, the steroidal scaffold was avoided when choosing the hydrophobic core because of the potential affinities of steroidal structures for various steroidal receptors. As for metyrapone, the nicotinoyl moiety occupies the same space as the steroidal C-ring. Since pyridyl as C-ring leads to a total loss of CYP17 inhibition,[13d] the nicotinoyl moiety was replaced by a biphenyl moiety furnished with polar substituents. Moreover, a substituted methylene bridge, observed for these three inhibitors, was sustained, as we regard it to be crucial for inhibition of the two enzymes. Furthermore, inspired by the

Table 1. Inhibition of CYP17 by Compounds **1**−**4**



Ref. 1 - 2, 1 - 4

compd	R^1	R^2	heterocycles	IC_{50} (nM)[b]
ref 1	Et	OMe	1-Im	> 5000
1	Et	OMe	3-Py	> 5000
2	Et	OMe	4-Py	1610
ref 2[c]	H	OH	1-Im	> 5000
3	H	OH	3-Py	4040
4	H	OH	4-Py	248
MYP[a]				> 10000
KTZ[a]				2780
ABT[a]				72

[a] MYP: metyrapone. KTZ: ketoconazole. ABT: abiraterone. [b] Concentration of inhibitors required to give 50% inhibition. The given values are mean values of at least three experiments. The deviations were within ±10%. The assay was run with human CYP17 expressed in *E.coli* using progesterone as substrate (25 μM). [c] **ref 2** was taken from ref 13a, where the inhibitory potency was tested with human testicular microsomes as 0.31 μM.

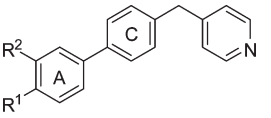
facts that pyridyl has already been successfully employed in other types of CYP17 inhibitors,[12a,b] leading to compounds being more potent than the corresponding imidazoles,[12a] and importantly that the pyridyl group is a common feature of CYP17 and CYP11B1 inhibitors, we replaced imidazole by pyridine. Accordingly, by use of this segmentation and hybridization procedure, biphenylmethylene pyridines were designed. Depending on the substituents at the core, these new compounds could be dual inhibitors of CYP17 and CYP11B2 or selective CYP17 inhibitors. This is because maximization of CYP17 inhibition was pursued as the first priority during the segment selection by choosing biphenyl instead of nicotinoyl. High inhibitory potency toward this enzyme should be the result, whereas inhibition toward CYP11B1 will largely depend on the substituents at the core.

As the first step, the effects of this design strategy on CYP17 inhibition were substantiated by comparing the imidazole and 3- and 4-pyridine analogues, i.e., **ref 1**,[13f] **ref 2**,[13a] and compounds **1**−**4** (Table 1). Encouraged by the positive results, more modifications on the A-ring were performed by introducing groups with different electrostatic potential, H-bond forming properties, and various steric demand to improve inhibition toward CYP17 and CYP11B1 (**5**−**25**, Tables 2 and 3). After determination of CYP17 inhibition, the most potent compounds were further tested for CYP11B1 inhibition and for selectivity against CYP11B2, CYP19, and hepatic CYP3A4.

Results

Chemistry. The synthesis of compounds **1**−**25** is shown in Schemes 1 and 2. A general strategy was employed starting from the corresponding (4-bromophenyl)pyridylmethanones, which are commercially available but were easily prepared from bromobenzene and the corresponding nicotinic or isonicotinic acid via Friedel−Crafts acylation. The starting material and the corresponding boronic acids were first reacted via Suzuki coupling to form the biaryl moieties, and then the ketone group was transferred by Wolff−Kishner reduction to afford the final methylene products. Subsequently, a more

Table 2. Inhibition of CYP17 by Compounds **5**−**20**

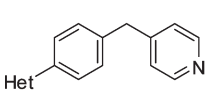


5 - 20

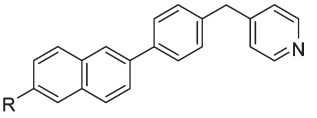
compd	R^1	R^2	IC_{50} (nM)[b]
5	NH_2	H	408
6	H	NH_2	226
7	NH_2	NH_2	337
4	OH	H	248
8	H	OH	97
9	OH	OH	52
10	OH	F	186
11	AcNH	H	> 5000
12	H	AcNH	876
13	NH_2CO	H	1790
14	F	H	386
15	F	F	598
16	OMe	F	3340
17	BocNH	H	1370
18	BocNH	F	> 10000
19	BocNH	BocNH	> 10000
20	OMe	BocNH	> 10000
MYP[a]			> 10000
KTZ[a]			2780
ABT[a]			72

[a] MYP: metyrapone. KTZ: ketoconazole. ABT: abiraterone. [b] Concentration of inhibitors required to give 50% inhibition. The given values are mean values of at least three experiments. The deviations were within ±10%. The assay was run with human CYP17 expressed in *E.coli* using progesterone as substrate (25 μM).

Table 3. Inhibition of CYP17 by Compounds **21**−**25**



21 - 23



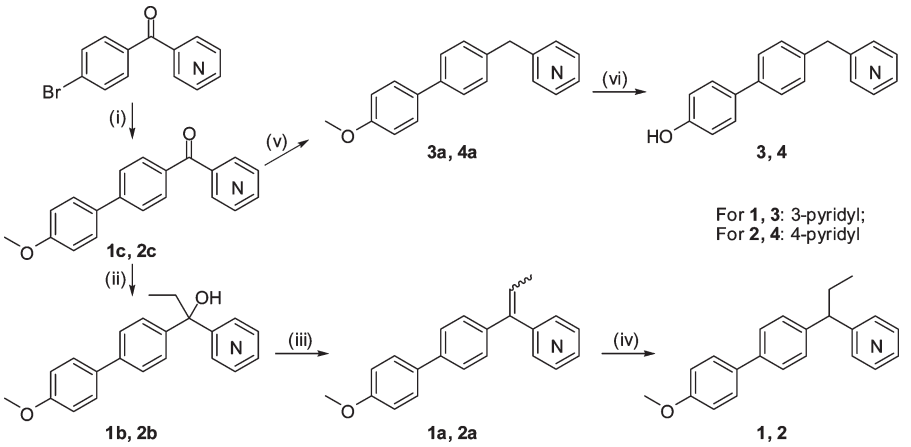
24 - 25

compd	heterocycle	R	IC_{50} (nM)[b]
21	2-thiophene		577
22	3-thiophene		647
23	5-indole		760
24		OMe	2000
25		OH	438
MYP[a]			> 10000
KTZ[a]			2780
ABT[a]			72

[a] MYP: metyrapone. KTZ: ketoconazole. ABT: abiraterone. [b] Concentration of inhibitors required to give 50% inhibition. The given values are mean values of at least three experiments. The deviations were within ±10%. The assay was run with human CYP17 expressed in *E.coli* using progesterone as substrate (25 μM).

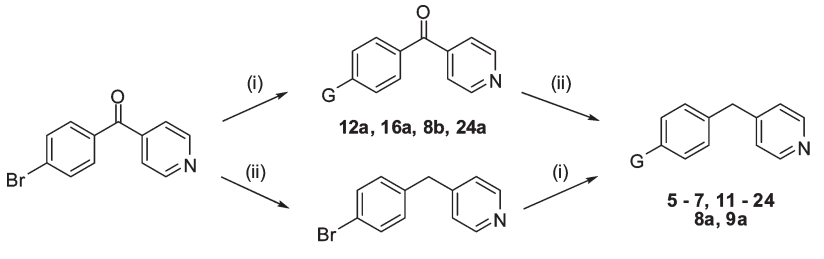
efficient strategy was used by first synthesizing the 4-bromobenzylpyridine **5a** as a common building block, followed by introduction of the aryl ring via Suzuki coupling. For compounds **1** and **2** substituted with an ethyl on the methylene bridge, the ketone intermediates were reacted with ethylmagnesium bromide to give the corresponding alcohols **1b** and **2b**. After H_2O elimination in formic acid using $Pd(OAc)_2$ as catalyst, hydrogenation was performed to yield the final compounds. Moreover, the OH analogues **8**, **9**, **10**,

Scheme 1[a]



[a] Reagents and conditions. (i) Method A: $Pd(OAc)_2$, 4-methoxyphenylboronic acid, Na_2CO_3, TBAB, toluene, H_2O, ethanol, reflux, 6 h. (ii) Method C: EtMgCl, THF. (iii) Method E: $Pd(OAc)_2$, HCOOH, reflux, 16 h. (iv) Method F: Pd/C, H_2. (v) Method B: N_2H_4, KOH, ethylene glycol, reflux, 4 h. (vi) Method D: BBr_3, DCM.

Scheme 2[a]



Comp	R^1	R^2	Comp	R^1	R^2
5	NH_2	H	14	F	H
6	H	NH_2	15	F	F
7	NH_2	NH_2	16	OMe	F
8a, 8b	H	OMe	17	BocNH	H
9a	OMe	OMe	18	BocNH	F
11	AcNH	H	19	BocNH	BocNH
12, 12a	H	AcNH	20	BocNH	OMe
13	NH_2CO	H			

[a] Reagents and conditions. (i) Method A: $Pd(OAc)_2$, corresponding boronic acid, Na_2CO_3, TBAB, toluene, H_2O, ethanol, reflux, 6 h. (ii) Method B: N_2H_4, KOH, ethylene glycol, reflux, 4 h.

and **25** were obtained by ether cleavage of the corresponding methoxy compounds **8a**, **9a**, **16**, and **24**, respectively, using boron tribromide.

CYP17 Inhibition. Inhibitory activities of the synthesized compounds for CYP17 were determined using the 50 000 sediment after homogenation of *E.coli* expressing human CYP17 as well as cytochrome P450 reductase.[17a] The assay was run with progesterone as substrate at high concentrations of 25 μM and NADPH as cofactor.[13c] Separation of substrate and product was accomplished by HPLC using UV detection. IC_{50} values are presented in comparison to ketoconazole and abiraterone in Tables 1−3.

To validate the possible benefits achieved after replacement of imidazolyl by pyridyl, the inhibitory potencies of imidazole, 3-pyridine, and 4-pyridine analogues were compared. The SARs obtained from our previous work on biphenylmethylene imidazoles confirm that (a) an ethyl group substituted on the methylene bridge elevates the inhibitory potency and (b) H-bond forming groups such as hydroxy at the 4-position of the A-ring increase the activity.[13d−g] Taking these SARs into consideration, reference compounds **ref 1**[13f] and **ref 2**[13a] were chosen, each possessing one advantageous structural feature, ethyl or hydroxy, but showing low activity. Accordingly, the 3- and 4-pyridyl analogues were synthesized (Table 1). It becomes apparent that no matter which substituent was furnished, the 3-pyridine analogues **1** and **3** showed similar inhibitory potencies compared to the reference compounds ($IC_{50} \geq 5000$ nM). However, the 4-pyridine analogue with an ethyl substituent on the methylene bridge exhibited an IC_{50} value of 1610 nM, thus being more potent than the corresponding imidazole analogue **ref 1** ($IC_{50} > 5000$ nM). The most active compound was the 4-pyridine analogue **4** furnished with hydroxy at the 4-position of the A-ring, showing an IC_{50} value of 248 nM.

Encouraged by these results, more compounds were designed on the basis of the biphenylmethylene 4-pyridine scaffold (Table 2). It can be seen that the substituents at

the A-ring showed a significant influence on the inhibitory potencies. The 4-amino substituted compound **5** showed an IC_{50} value of 408 nM, whereas its 3-amino analogue **6** was even more potent (IC_{50} = 226 nM). Interestingly, the 3,4-diamino compound **7** exhibited potent activity with an IC_{50} value in between (337 nM). Moreover, the hydroxy analogues were more potent than the amino derivatives. The 3-OH compound **8** was 3-fold more potent (IC_{50} = 97 nM) compared to its corresponding NH_2 analogue and to the 4-OH compound **4** (IC_{50} = 248 nM). The 3,4-di-OH compound **9** turned out to be the most potent one in this series with an IC_{50} value of 52 nM, being even more potent than abiraterone (IC_{50} = 72 nM). The introduction of fluorine in the 3-position of compound **4**, resulting in compound **10**, increased activity to 186 nM. The phenomenon that 3-substituted analogues are more potent than the corresponding 4-substituted compounds was also observed with the 4-acetamido compound **11** and its 3-analogue **12** (IC_{50} = 876 nM). The retroamide **13** (IC_{50} = 1790 nM) showed a slightly increased potency compared to compound **11**. As for fluorine derivatives, introduction of additional F resulted in loss of inhibitory potency (**15**), which is in accordance with our previous findings,[13g] while the compound with a single fluorine in the 4-position of the A-ring (**14**) exhibited strong inhibition (IC_{50} = 386 nM). Another interesting observation was the little activity of compounds **17**−**20**, indicating that the Boc-amido group is not appropriate for highly active compounds, no matter what is introduced at the 3- or 4-position.

Finally, the A-ring phenyl group was exchanged by heterocycles and substituted naphthalenes (Table 3). The thiophene (**21** and **22**) and indole (**23**) compounds showed modest activity with IC_{50} values around 600 nM. For the naphthalene analogues, the great influence of the substituent on the A-ring was again observed. The hydroxy derivative **25** showed rather potent inhibition (IC_{50} = 438 nM), while the corresponding methoxy derivative **24** was less active.

CYP11B1 Inhibition. After it has been shown that the replacement of imidazolyl by pyridyl significantly increased the CYP17 inhibition, selected compounds (**4**−**10**, **12**, **14**, **15**, **21**−**23**, and **25**) were examined for their CYP11B1 inhibition (Table 4). The inhibitory activities were determined in V79 MZh cells expressing human CYP11B1.[17b,c] The V79 MZh cells were incubated with [^{14}C]deoxycorticosterone as substrate and the inhibitor in different concentrations. Product formation was monitored by HPTLC using a phosphoimager. It becomes apparent that the substituents on the A-ring also show profound influence on the inhibition of this enzyme. Compounds **4** and **6** exhibited rather strong effects with IC_{50} values of around 250 nM. Compounds **9** and **15** showed only weak or no inhibition (IC_{50} values of 1400 and 4742 nM, respectively), thus being rather selective inhibitors of CYP17, whereas the rest of the compounds **5**, **7**, **8**, **10**, **12**, **14**, **15**, **21**−**23**, and **25** showed modest inhibition values between 300 and 930 nM.

Selectivity. The inhibition of CYP11B2 by selected potent compounds was determined as well using V79 MZh cells expressing human CYP11B2 (Table 4). CYP11B2 is the crucial enzyme responsible for the final steps in aldosterone biosynthesis (Chart 1). Inhibition of CYP11B2 could cause hyponatremia, hyperkalemia, and a series of recessive disorders, such as adrenal hyperplasia and hypovolemic shock.[18a] Selectivity over CYP11B2 is difficult to reach especially for the compounds inhibiting CYP11B1 because of the very high homology between these two enzymes of 93%. It can be seen that although compounds **8**, **10**, and **15** exhibited strong inhibition toward CYP11B2 (IC_{50} values of around 250 nM), some compounds (**4**, **5**, **7**, **12**, **14**, and **21**−**23**) showed modest inhibition with IC_{50} values ranging from 300 to 800 nM, whereas other compounds (**6**, **9**, and **25**) were very selective with IC_{50} values around 1000 nM. Important is that compound **6** as a dual inhibitor of CYP17 and CYP11B1, exhibiting a selectivity factor of 3.2 between CYP11B1 and CYP11B2.

Table 4. Inhibition of CYP11B1, CYP11B2, CYP19, and CYP3A4 by Selected Compounds

	IC_{50} (nM)[b]			
compd	CYP11B1[c]	CYP11B2[c]	CYP19[d]	CYP3A4[e]
4	251	341	3070	3210
5	522	406	24500	2450
6	287	921	2830	1520
7	902	367	>25000	nd[a]
8	342	261	663	538
9	1400	948	2440	7580
10	850	210	2693	896
12	307	567	nd[a]	nd[a]
14	928	515	nd[a]	668
15	4742	273	nd[a]	nd[a]
21	422	331	nd[a]	nd[a]
22	415	796	nd[a]	nd[a]
23	469	231	nd[a]	nd[a]
25	627	1130	nd[a]	1140
MYP[a]	15	72	>5000	nd[a]
KTZ[a]	127	67	>5000	57
ABT[a]	1610	1750	>5000	2700

[a] MYP: metyrapone. KTZ: ketoconazole. ABT: abiraterone. nd: not determined. [b] Standard deviations were within ±5%. All the data are the mean values of at least three tests. [c] Hamster fibroblasts expressing human CYP11B1 or CYP11B2 are used with deoxycorticosterone as the substrate at 100 nM. [d] Human placental CYP19 is used with androstenedione as the substrate at 500 nM. [e] Recombinantly expressed enzyme from baculovirus-infected insect microsome is used with 7-benzyloxytrifluoromethyl coumarin as the substrate at 50 μM.

Furthermore, the selectivity of these potent compounds toward CYP19 and hepatic CYP3A4 has also been tested as a criterion for safety (Table 4). CYP19 is a unique enzyme catalyzing the peripheral conversion of androgens to estrogens by hydroxylation and subsequent elimination of the C19 methyl group, resulting in steroid A-ring aromatization. Estrogen deficiency causes osteoporosis, increased fracture risk,[18b] and memory loss.[18c] Under CYP17 inhibition, there is a reduction of the estrogen plasma concentrations because the androgens, as substrates for estrogen formation, are decreased. A further reduction of estrogen levels by CYP19 inhibition would be detrimental. As can be seen, all the tested compounds except **8** showed no inhibition of CYP19 (IC_{50} values above 2000 nM).

The important role of CYP3A4 in drug metabolism and drug−drug interaction has been addressed, and the compounds have been tested for inhibition of this enzyme as well. Most of them showed weak or no inhibition of CYP3A4 (IC_{50} > 1000 nM). Compounds **4** and **9** exhibited a better selectivity than abiraterone (IC_{50} values of 3210 and 7580 nM vs 2700 nM).

Discussion and Conclusion

The design concept applied in the present paper using the steroidal CYP17 inhibitor abiraterone and the CYP11B1 inhibitor metyrapone and our experience in nonsteroidal

biphenylmethylene based CYP17 inhibitors (including **1K**) was successful. Exchange of the imidazolyl moiety by a 4-pyridyl rest led (depending on the substitution pattern at the A-ring) on the one hand to compound **9**, exceeding abiraterone in activity and selectivity, and on the other hand to compound **6**, a dual inhibitor of CYP17 and CYP11B1.

The elevated inhibition of CYP17 caused by the novel pyridines compared to the corresponding imidazoles might be due to the prolonged distance between the sp^2 hybrid N and the methylene C. This elongation of the molecule places the H-bond forming groups at the A-ring closer to the amino acid residues, facilitating better H-bond formation. Moreover, it has been found that substituents on the A-ring showed profound influence on the inhibitory potency of both CYP17 and CYP11B1. Hydrogen bond forming groups, like OH and NH_2, significantly elevated CYP17 inhibition, probably because of the interaction with Arg109, Lys231, His235, and Asp298, similar to what we described recently.[13f] This finding indicates that imidazoles and pyridines may adopt the same binding mode in the enzyme pocket. Among them, OH analogues were found to be more potent than the corresponding NH_2 analogues (e.g., compounds **4** and **5** (IC_{50} values of 248 and 408 nM), compounds **8** and **6** (IC_{50} values of 97 and 226 nM), and compounds **9** and **7** (IC_{50} values of 52 and 337 nM)), probably because the hydrogen bonds formed by O are stronger than the ones formed by N.[19] Interestingly, meta-substituted analogues are more potent than the corresponding para-analogues. After methylation of OH (compounds **16** and **20**) or acylation of NH_2 (**11**, **12**, **17**−**20**), the inhibitory potency dropped along with the augment of bulk, which is probably due to the steric hindrance with His235, Arg109, and the proximal I-helix residues as described previously.[13f]

Regarding the additional CYP11B1 inhibition we were aiming at in this study, the exchange of the nicotinoyl moiety from metyrapone (because it leads to a loss of CYP17 inhibition) by a biphenyl moiety with small polar substituents was successful and resulted in compounds with dual, CYP17 and CYP11B1, inhibition. It was found that compounds **4**−**6**, **8**, and **12** with one single H-bond donor showed potent to modest inhibition (IC_{50} values ranged from 250 to 350 nM, with compound **5** as an exception showing IC_{50} value of 522 nM), whereas H-bond acceptors or two donors resulted in rather weak inhibitors of CYP11B1 (compounds **7**, **9**, and **10**; IC_{50} values of greater than 800 nM). Since these compounds with differing CYP11B1 inhibition are potent CYP17 inhibitors, they are accordingly either dual inhibitors of CYP17/11B1 or selective CYP17 inhibitors.

The A-ring substituents also diversified the inhibitory potency toward the nontarget enzyme CYP11B2. It was found that compounds with strong CYP11B1 inhibition were also associated with CYP11B2 inhibition. This is not surprising, since homology between these two enzymes is more than 93%. As inhibition of CYP11B2 has to be avoided, the most interesting dual inhibitor in this study is compound **6** showing a selectivity factor of 3.2 regarding CYP11B1 and CYP11B2. For the application as a dual CYP17/CYP11B1 inhibitor, this selectivity toward CYP11B2 is certainly not sufficient. However, compound **6** should be an interesting lead for further optimization. Since highly active and selective CYP11B2 inhibitors with in vivo activity[15a] reaching selectivity factors of 1000[15b] have been identified by our group for the treatment of congestive heart failure and myocardial fibrosis, we are confident that the selectivity of compound **6** can be further improved. Structure modifications are presently being performed.

The use of selective multitarget-directed ligands has already been proposed for the treatment of other diseases to enhance efficacy and to improve safety, for example, agents inhibiting angiotensin converting enzyme (ACE) and neutral endopeptidase (NEP) in the treatment of hypertension, multikinase inhibitors (MKI) with combined inhibition of vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF) for cancer therapy, and dual binding site acetylcholinesterase inhibitors (AChEI) for Alzheimer's disease.[20] Compared to the traditional combinational application of two or more drugs, multitarget-directed agents can reduce the risk of drug−drug interactions and achieve better compliance. The conception of selective dual inhibition of CYP17 and CYP11B1 is a novel treatment for PC. There is indeed a high medical need in prostate cancer patients to combat elevated glucocorticoid levels and to prevent stimulation of mutated androgen receptors by glucocorticoids. CYP11B1 catalyzing the last step in cortisol biosynthesis is an ideal target to decrease corticosterone and cortisol production. However, there are no highly selective inhibitors of this enzyme described so far that could be used in a combination therapy with CYP17 inhibitors. Metyrapone is a compound not selective enough to inhibit other steroidogenic CYP enzymes and therefore shows severe side effects. Accordingly it is not an appropriate candidate for this purpose, although it has been used in the treatment of Cushing's syndrome for a long time. Another unselective compound, the antimycotic ketoconazole, has already been employed to treat prostate cancer patients with ectopic adrenocorticotropic hormone syndrome[8d] because it inhibits both androgen and corticosteroid (not only glucocorticoid but also mineralocorticoid) biosynthesis. However, the response was not satisfactory because of the weak potency of ketoconazole (CYP17 IC_{50} = 2780 nM). Compound **6** should be a much better candidate than ketoconazole showing a good dual inhibition of CYP17 and CYP11B1 with IC_{50} values around 200 nM for both enzymes, a 3-fold selectivity of CYP11B1 over CYP11B2, and nearly no inhibition of CYP19 and CYP3A4. Because selectivity for CYP11B2 should be further enhanced, compound **6** could be an ideal candidate for this optimization process.

Importantly, compound **9** showed excellent selectivity with almost no inhibition compared with all other enzymes tested. It is highly active showing an IC_{50} value of 52 nM. As in our experimental setup with high substrate concentrations, this value has to be ranked higher than similar IC_{50} values obtained by other groups using much lower substrate concentrations. The fact that compound **9** not only shows strong inhibition of androgen formation (CYP17) but also does not block androgen conversion to estrogens (CYP19) provides maximally low androgen levels (a prerequisite for the therapeutic success). After validation in vivo, this compound could be a promising drug candidate for further development.

On the basis of the results of the present study, we propose a novel strategy for the treatment of prostate cancer: CYP17 inhibitors with different selectivity profiles according to the status of the patients should be applied. For normal patients, selective CYP17 inhibitors that do not interfere with other steroidogentic CYPs should be used to avoid side effects, whereas for patients with mutated androgen receptors or ectopic adrenocorticotropic hormone syndrome, dual inhibitors of CYP17 and CYP11B1 are the best choice for personalized medicine.

Experimental Section

CYP17 Preparation and Assay. Human CYP17 was expressed in *E. coli*[17a] (coexpressing human CYP17 and NADPH-P450 reductase), and the assay was performed as previously described.[13c]

Inhibition of Hepatic CYP3A4. The recombinantly expressed enzyme from baculovirus-infected insect microsomes (supersomes) was used, and the manufacturer's instructions (www.gentest.com) were followed.

Inhibition of CYP11B1 and CYP11B2. V79MZh cells expressing human CYP11B1 or CYP11B2 were incubated with [^{14}C]11-deoxycorticosterone as substrate. The assay was performed as previously described.[17b,c]

Inhibition of CYP19. The inhibition of CYP19 was determined in vitro using human placental microsomes with [1β-^{3}H]-androstenedione as substrate.[17d]

Chemistry Section. General. Melting points were determined on a Mettler FP1 melting point apparatus and are uncorrected. IR spectra were recorded neat on a Bruker Vector 33FT infrared spectrometer. ^{1}H NMR spectra were measured on a Bruker DRX-500 (500 MHz). Chemical shifts are given in parts per million (ppm), and TMS was used as an internal standard for spectra. All coupling constants (J) are given in Hz. ESI (electrospray ionization) mass spectra were determined on a TSQ quantum (Thermo Electron Corp.) instrument. The purities of the final compounds were controlled by a Surveyor LC system. Purities were greater than 95%. Column chromatography was performed using silica gel 60 (50−200 μm), and reaction progress was determined by TLC analysis on Alugram SIL G/UV$_{254}$ (Macherey-Nagel). Boronic acids and bromoaryls used as starting materials were commercially obtained (CombiBlocks, Chempur, Aldrich, Acros).

Method A: Suzuki Coupling. The corresponding brominated aromatic compound (1 equiv) was dissolved in toluene (7 mL/mmol), and an aqueous 2.0 M Na_2CO_3 solution (3.2 mL/mmol), an ethanolic solution (3.2 mL/mmol) of the corresponding boronic acid (1.5−2.0 equiv), and tetrabutylammonium bromide (1 equiv) were added. The mixture was deoxygenated under reduced pressure and flushed with nitrogen. After this cycle was repeated several times, $Pd(OAc)_2$ (5 mol %) was added and the resulting suspension was heated under reflux for 2−6 h. After the mixture was cooled, ethyl acetate (10 mL) and water (10 mL) were added and the organic phase was separated. The water phase was extracted with ethyl acetate (2 × 10 mL). The combined organic phases were washed with brine, dried over Na_2SO_4, filtered over a short plug of Celite, and evaporated under reduced pressure. The compounds were purified by flash chromatography on silica gel.

4′-(Pyridin-4-ylmethyl)biphenyl-4-amine (5). 5 was synthesized according to method A using **5a** (0.35 g, 1.41 mmol) and 4-aminophenylboronic acid (0.29 g, 2.12 mmol). Yield, 0.32 g (87%); white solid; mp 217−218 °C; R_f = 0.19 (DCM/MeOH, 20:1); δ_H (CD_3OD, 500 MHz) 4.04 (s, 2H), 6.77 (d, J = 8.5 Hz, 2H), 7.23 (d, J = 8.1 Hz, 2H), 7.29 (d, J = 5.9 Hz, 2H, Py 2,6-H), 7.36 (d, J = 8.5 Hz, 2H), 7.48 (d, J = 8.2 Hz, 2H), 8.40 (d, J = 6.1 Hz, 2H, Py 3,5-H); MS (ESI) m/z = 261 [M$^+$ + H].

4′-(Pyridin-4-ylmethyl)biphenyl-3-amine (6). 6 was synthesized according to method A using **5a** (0.30 g, 1.21 mmol) and 3-aminophenylboronic acid (0.25 g, 1.81 mmol). Yield, 0.26 g (83%); white solid; mp 79−80 °C; R_f = 0.45 (DCM/MeOH, 95:5); δ_H ($CDCl_3$, 500 MHz) 4.00 (s, 2H), 5.13 (s, 2H), 6.54 (dd, J = 2.2, 7.9 Hz, 1H), 6.74 (dd, J = 1.6, 7.6 Hz, 1H), 6.81 (t, J = 1.9 Hz, 1H), 7.07 (t, J = 7.9 Hz, 1H), 7.27 (d, J = 5.9 Hz, 2H), 7.30 (d, J = 8.2 Hz, 2H), 7.49 (d, J = 7.9 Hz, 2H), 8.46 (d, J = 5.9 Hz, 2H); MS (ESI) m/z = 261 [M$^+$ + H].

4′-(Pyridin-4-ylmethyl)biphenyl-3,4-diamine (7). 7 was synthesized according to method A using **5a** (0.35 g, 1.41 mmol) and 3,4-diaminophenylboronic acid (0.32 g, 2.11 mmol). Yield, 0.32 g (83%); white solid; mp 180−181 °C; R_f = 0.15 (DCM/MeOH, 20:1); δ_H ($CDCl_3$, 500 MHz) 3.48 (s, br, 4H, NH_2), 3.98 (s, 2H, CH_2), 6.76 (d, J = 8.2 Hz, 1H,), 6.92−6.96 (m, 2H), 7.13 (d, J = 6.0 Hz, 2H), 7.18 (d, J = 8.2 Hz, 2H), 7.47 (d, J = 8.2 Hz, 2H), 8.50 (dd, J = 1.6, 6.0 Hz, 2H); MS (ESI) m/z = 276 [M$^+$ + H].

4-[(3′,4′-Dimethoxybiphenyl-4-yl)methyl]pyridine (9a). 9a was synthesized according to method A using **5a** (0.31 g, 1.25 mmol) and 3,4-dimethoxyphenylboronic acid (0.34 g, 1.88 mmol). Yield, 0.34 g, (90%). This compound was used directly in the next step without further purification and characterization.

4′-(Pyridin-4-ylmethyl)biphenyl-4-carboxamide (13). 13 was synthesized according to method A using **5a** (0.25 g, 1.01 mmol) and 4-carbamoylphenylboronic acid (0.25 g, 1.51 mmol). Yield, 0.26 g (89%); white solid; mp 241−242 °C; R_f = 0.21 (DCM/MeOH, 50:1); δ_H (CD_3OD, 500 MHz) 4.09 (s, 2H,CH_2), 7.09−7.11 (m, 4H), 7.64 (d, J = 7.9 Hz, 2H), 7.71 (d, J = 7.8 Hz, 2H), 7.94 (d, J = 8.1 Hz, 2H), 8.42 (d, J = 4.7 Hz, 2H, Py 3,5-H); MS (ESI) m/z = 289 [M$^+$ + H].

4-[(4′-Fluorobiphenyl-4-yl)methyl]-pyridine (14). 14 was synthesized according to method A using **5a** (301 mg, 1.21 mmol) and 4-fluorophenylboronic acid (254 mg, 1.82 mmol). Yield, 168 mg (53%); white solid; mp 114−115 °C; R_f = 0.49 (DCM/MeOH, 95:5) δ_H ($CDCl_3$, 500 MHz) 3.96 (s, 2H), 7.03−7.07 (m, 2H), 7.12 (d, J = 5.4 Hz, 2H), 7.17 (d, J = 8.5 Hz, 2H), 7.43 (d, J = 8.2 Hz, 2H), 7.44−7.47 (m, 2H), 8.45 (d, J = 5.3 Hz, 2H); MS (ESI) m/z = 264 [M$^+$ + H]

4-[(3′,4′-Difluorobiphenyl-4-yl)methyl]pyridine (15). 15 was synthesized according to method A using **5a** (0.125 g, 0.5 mmol) and 3,4-difluorophenylboronic acid (0.21 g, 0.75 mmol). Yield, 0.12 g (87%); colorless oil; R_f = 0.27 (hexane/EtOAc, 1:1); δ_H ($CDCl_3$, 500 MHz) 3.93 (s, 2H), 7.05 (d, J = 6.1 Hz, 2H), 7.10−7.22 (m, 4H), 7.26−7.31 (m, 1H), 7.39 (d, J = 8.2 Hz, 2H), 8.44 (d, J = 6.1 Hz, 2H); MS (ESI) m/z = 282 [M$^+$ + H].

***tert*-Butyl 4′-(Pyridin-4-ylmethyl)biphenyl-4-ylcarbamate (17). 17** was synthesized according to method A using **5a** (0.30 g, 1.21 mmol) and 4-(*tert*-butoxycarbonylamino)phenylboronic acid (0.43 g, 1.81 mmol). Yield, 0.38 g (87%); white solid; mp 198−199 °C; R_f = 0.26 (DCM/MeOH, 50:1); δ_H (CD_3OD, 500 MHz) 1.53 (s, 9H, Boc), 4.05 (s, 2H, CH_2), 7.28−7.29 (m, 4H), 7.51−7.53 (m, 6H), 8.40 (d, J = 4.9 Hz, 2H); MS (ESI) m/z = 361 [M$^+$ + H].

4-[4-(Thiophen-2-yl)benzyl]pyridine (21). 21 was synthesized according to method A using **5a** (0.35 g, 1.41 mmol) and thiophen-2-ylboronic acid (0.27 g, 2.12 mmol). Yield, 0.32 g (91%); white solid; mp 82−83 °C; R_f = 0.26 (DCM/MeOH, 50:1); δ_H (CD_3OD, 500 MHz) 4.00 (s, 2H, CH_2), 7.05 (dd, J = 4.0, 4.6 Hz, 1H), 7.22 (d, J = 7.2 Hz, 2H), 7.26 (d, J = 4.6 Hz, 2H), 7.33 (d, J = 4.7 Hz, 2H), 7.56 (d, J = 7.9 Hz, 2H), 8.40 (d, J = 3.9 Hz, 2H); MS (ESI) m/z = 252 [M$^+$ + H].

4-[4-(Thiophen-3-yl)benzyl]pyridine (22). 22 was synthesized according to method A using **5a** (0.35 g, 1.41 mmol) and thiophen-3-ylboronic acid (0.27 g, 2.12 mmol). Yield, 0.31 g (90%); white solid; mp 97−98 °C; R_f = 0.26 (DCM/MeOH, 50:1); δ_H (CD_3OD, 500 MHz) 4.03 (s, 1H, CH_2), 7.24 (d, J = 8.2 Hz, 2H), 7.28 (d, J = 6.0 Hz, 2H), 7.43−7.45 (m, 2H), 7.60−7.63 (m, 3H), 8.40 (d, J = 6.45 Hz, 2H); MS (ESI) m/z = 252 [M$^+$ + H].

5-[4-(Pyridin-4-ylmethyl)phenyl]-1*H*-indole (23). 23 was synthesized according to method A using **5a** (0.30 g, 1.21 mmol) and 1*H*-indol-5-ylboronic acid (0.27 g, 2.12 mmol). Yield, 0.30 g (87%); white solid; mp 170−171 °C; R_f = 0.23 (DCM/MeOH, 20:1); δ_H (CD_3OD, 500 MHz) 4.04 (s, 2H, CH_2), 6.48 (d, J = 3.7 Hz, 1H), 7.25−7.27 (m, 3H), 7.30 (d, J = 5.9 Hz, 2H), 7.35−7.37 (m, 1H), 7.42 (d, J = 8.5 Hz, 2H), 7.58 (d, J = 8.2 Hz, 2H), 7.76−7.78 (m, 1H), 8.40 (d, J = 5.9 Hz, 2H); MS (ESI) m/z = 285 [M$^+$ + H].

Method B: Wolff−Kishner Reduction. To an ice-cooled solution of the appropriate ketone (10 mmol) in ethylene glycol (100 mL) were added hydrazine hydrate (70 mmol) and potassium hydroxide (10 mmol). Then the resulting mixture was heated to 150 °C for 1 h. After the mixture was cooled, a further batch of potassium hydroxide (50 mmol) was added, and the

mixture was heated to 210 °C for 2 h. After cooling to ambient temperature, it was diluted with water (300 mL), and the resulting mixture was extracted with ethyl acetate (3 × 50 mL). The combined organic phases were washed with brine, dried over $MgSO_4$, and evaporated under reduced pressure. Then the desired product was purified by chromatography on silica gel.

4-(4-Bromobenzyl)pyridine (5a). 5a was synthesized according to method B using (4-bromophenyl)pyridin-4-ylmethanone (0.39 g, 1.5 mmol). Yield, 0.21 g (52%); amber oil; R_f = 0.35 (PE/EtOAc, 10:1); δ_H ($CDCl_3$, 500 MHz) 3.89 (s, 2H, CH_2), 7.02 (d, J = 8.3 Hz, 2H), 7.05 (d, J = 5.9 Hz, 2H), 7.41 (d, J = 8.3 Hz, 2H), 8.48 (d, J = 5.9 Hz, 2H); δ_C ($CDCl_3$, 125 MHz) 40.5 (CH_2), 120.5, 124.0, 130.6, 131.7, 137.7, 149.8; MS (ESI) m/z = 249 [M^+ + H].

***N*-(4′-Isonicotinoylbiphenyl-3-yl)acetamide (12). 12** was synthesized according to method B using **12a** (0.32 g, 1.0 mmol). Yield, 0.19 g (62%); yellowish solid; mp 123−124 °C; R_f = 0.27 (DCM/MeOH, 95:5); δ_H (DMSO-d_6, 500 MHz) 2.06 (s, 3H, CH_3), 4.00 (s, 2H, CH_2), 7.27 (d, J = 5.9 Hz, 2H), 7.29 (t, J = 1.9 Hz, 1H), 7.32−7.38 (m, 3H), 7.52−7.57 (m, 3H), 7.88 (t, J = 1.9 Hz, 1H), 8.47 (d, J = 5.9 Hz, 2H), 10.01 (s, 1H); MS (ESI) m/z = 303 [M^+ + H].

4-[(3′-Fluoro-4′-methoxybiphenyl-4-yl)methyl]pyridine (16). 16 was synthesized according to method B using **16a** (0.43 g, 1.38 mmol). Yield, 0.37 g (91%); white solid; mp 98−99 °C; R_f = 0.46 (hexane/EtOAc, 1:1); δ_H ($CDCl_3$, 500 MHz) 3.84 (s, 3H), 3.91 (s, 2H), 6.93 (t, J = 8.5 Hz, 1H), 7.05 (d, J = 6.3 Hz, 2H), 7.14 (d, J = 7.9 Hz, 2H), 7.18−7.26 (m, 1H), 7.21 (s, 1H), 7.39 (d, J = 7.9 Hz, 2H), 8.43 (d, J = 6.3 Hz, 2H); MS (ESI) m/z = 294 [M^+ + H].

4-[4-(6-Methoxynaphthalen-2-yl)benzyl]pyridine (24). 24 was synthesized according to method B using **24a** (0.40 g, 1.18 mmol). Yield, 0.34 g (89%); white solid; R_f = 0.49 (DCM/MeOH, 95:5); δ_H ($CDCl_3$, 500 MHz) 3.89 (s, 3H), 4.02 (s, 2H), 7.18 (dd, J = 2.5, 8.8 Hz, 1H), 7.29 (d, J = 6.0 Hz, 2H), 7.34 (d, J = 2.5 Hz, 1H), 7.37 (d, J = 8.5 Hz, 2H), 7.73 (d, J = 8.2 Hz, 2H), 7.77 (dd, J = 1.9, 8.5 Hz, 1H), 7.88 (d, J = 8.8 Hz, 2H), 8.11 (d, J = 1.6 Hz, 1H), 8.47 (d, J = 6.0 Hz, 2H); MS (ESI) m/z = 326 [M^+ + H].

Method C: Grignard Reaction. Under exclusion of air and moisture a 1.0 M RMgBr (1.2 equiv) solution in THF was added dropwise to a solution of the aldehyde or ketone (1 equiv) in THF (12 mL/mmol). The mixture was stirred overnight at room temperature. Then ethyl acetate (10 mL) and saturated ammonium chloride solution (10 mL) were added and the organic phase was separated. The organic phase was extracted with water and brine, dried over Na_2SO_4, and evaporated under reduced pressure. The crude products were purified by flash chromatography on silica gel.

Method D: Ether Cleavage with BBr_3. To a solution of the corresponding ether (1 equiv) in DCM (5 mL/mmol) at −78 °C was added 1 M boron tribromide in DCM (5 equiv). The resulting mixture was stirred at room temperature for 16 h. Then water (25 mL) was added and the emulsion was stirred for further 30 min. The resulting mixture was extracted with ethyl acetate (3 × 25 mL). The combined organic phases were washed with brine, dried over Na_2SO_4, and evaporated under reduced pressure. Then the desired product was purified by chromatography on silica gel.

4-[(4′-Hydroxybiphenyl-4-yl)methyl]pyridine (4). 4 was synthesized according to method D using **4a** (0.38 g, 1.37 mmol) and 1 M BBr_3 solution in DCM (6.85 mL, 6.85 mmol). Yield, 0.39 g (10%); white solid; mp 224−225 °C; R_f = 0.24 (DCM/MeOH, 95:5); δ_H (DMSO-d_6, 500 MHz) 3.97 (s, 2H), 6.83 (d, J = 8.5 Hz, 2H), 7.25−7.30 (m, 4H), 7.45 (d, J = 8.5 Hz, 2H), 7.50 (d, J = 8.2 Hz, 2H), 8.46 (d, J = 5.9 Hz, 2H), 9.52 (s, 1H); MS (ESI) m/z = 262 [M^+ + H].

4-[(3′-Hydroxybiphenyl-4-yl)methyl]pyridine (8). 8 was synthesized according to method D using **8a** (0.17 g, 0.62 mmol) and 1 M BBr_3 solution in DCM (3.10 mL, 3.10 mmol). Yield, 0.10 g (62%); white solid; mp 153−154 °C; R_f = 0.41 (EtOAc/hexane, 4:1); δ_H (DMSO-d_6, 500 MHz) 3.99 (s, 2H), 6.74 (dd, J = 2.5, 8.2 Hz, 1H), 6.99 (t, J = 2.1 Hz, 1H), 7.04 (d, J = 7.9 Hz, 1H), 7.23 (t, J = 7.9 Hz 1H), 7.28 (d, J = 5.7 Hz, 2H), 7.31 (d, J = 8.2 Hz, 2H), 7.53 (d, J = 8.2 Hz, 2H), 8.47 (d, J = 5.7 Hz, 2H), 9.50 (s, 1H); MS (ESI) m/z = 262 [M^+ + H].

4′-(Pyridin-4-ylmethyl)biphenyl-3,4-diol (9). 9 was synthesized according to method D using **9a** (0.26 g, 0.85 mmol) and 1 M BBr_3 solution in DCM (4.20 mL, 4.20 mmol). Yield, 0.22 g (93%); white solid; R_f = 0.55 (DCM/MeOH, 10:1); δ_H (DMSO-d_6, 500 MHz) 3.97 (s, 2H), 6.79 (d, J = 8.2 Hz, 1H), 6.90 (dd, J = 2.2, 8.2 Hz, 1H), 7.00 (d, J = 2.2 Hz, 1H), 7.22−7.29 (m, 4H), 7.45 (d, J = 8.2 Hz, 2H), 8.46 (d, J = 6.0 Hz, 2H), 8.98 (s, 1H), 9.02 (s, 1H); MS (ESI) m/z = 278 [M^+ + H].

3-Fluoro-4′-(pyridin-4-ylmethyl)biphenyl-4-ol (10). 10 was synthesized according to method D using **16** (0.18 g, 0.64 mmol) and 1 M BBr_3 solution in DCM (3.20 mL, 3.20 mmol). Yield, 0.12 g (67%); white solid; mp 229−230 °C; R_f = 0.16 (DCM/MeOH, 19:1); δ_H (DMSO-d_6, 500 MHz) 3.98 (s, 2H), 7.00 (t, J = 8.8 Hz, 1H), 7.25−7.31 (m, 5H), 7.44 (dd, J = 2.2 Hz, $^2J_{HF}$ = 12.9 Hz, 1H), 7.55 (d, J = 8.5 Hz, 2H), 8.46 (d, J = 5.9 Hz, 2H), 9.95 (s, 1H); MS (ESI) m/z = 280 [M^+ + H].

6-[4-(Pyridin-4-ylmethyl)phenyl]naphthalen-2-ol (25). 25 was synthesized according to method D using **24** (0.33 g, 1.00 mmol) and 1 M BBr_3 solution in DCM (3.00 mL, 3.00 mmol). Yield, 0.25 g (80%); white solid; R_f = 0.49 (DCM/MeOH, 10:1); δ_H (DMSO-d_6, 500 MHz) 4.00 (s, 2H, CH_2), 7.11 (dd, J = 2.2, 8.8 Hz, 1H), 7.14 (d, J = 2.2 Hz, 1H), 7.27 (d, J = 6.0 Hz, 2H), 7.34 (d, J = 8.2 Hz, 2H), 7.68−7.70 (m, 3H), 7.74 (d, J = 8.8 Hz, 1H), 7.82 (d, J = 8.8 Hz, 1H), 8.04 (s, 1H), 8.46 (d, J = 6.0 Hz, 2H), 9.80 (s, 1H); MS (ESI) m/z = 312 [M^+ + H].

Method E: Dehydroxylation with HCOOH. Under exclusion of air and moisture the appropriate alcohol (1 mmol) was dissolved in formic acid (10 mL per mmol). $Pd(OAc)_2$ (1 mol %) was added, and the mixture was heated to reflux for 16 h. After the reaction mixture was cooled, formic acid was distilled off and the mixture was neutralized using saturated sodium bicarbonate solution. Then the mixture was extracted with ethyl acetate (3 × 10 mL), and the combined organic phases were washed with brine, dried over Na_2SO_4, evaporated to dryness, and purified using column chromatography.

Method F: Hydrogenation. A solution of the corresponding alkene (1 mmol) in dry THF (15 mL/mmol) was treated with 10% Pd on charcoal (15 mg). The reaction vessel was repeatedly evacuated and flushed with hydrogen gas and left to stir at room temperature for 3 h, pressurized with 1 bar of H_2. The resulting reaction mixture was filtered through a short plug of Celite, which was washed with THF (40 mL). The filtrates were concentrated with an oil pump and purified using column chromatography.

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Supporting Information Available: Synthesis procedures and characterization of compounds **1**−**3**, **11**, **18**−**20** and other intermediates as well as HPLC purities and ^{13}C NMR and IR spectra of other final compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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